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CLOSE TO THE PROBLEMS OF DISEASE

" WHAT can be more happily exciting ", asks Hans Zinsser,[1] " than to study a disease in all its natural manifestations ? " Earlier he had learned " the incomparable satisfaction that lies in the exploration of the unknown, even when the problem is a small one, and the application of reasoning, controlled imagination, and precision of technique to the study of natural phenomena ". But his life-work had also a deeper motivation than excitement or satisfaction. Stirred by the horror of a typhus epidemic in Serbia, he resolved : " I would never aspire to administrative power or prominence so long as I could remain close, heart and hands, to the problems of disease."

To anyone wrestling with these problems comes a variety of experiences. There are moments of exaltation when some exciting observation has been made, some experiment has succeeded, some flash of illumination has come, some important conclusion has been established. But he does not stay long on the mountain top. Most of his time is taken up in unremitting attention to detail. There are endless tests to be carried through, results to be checked and counterchecked, controls to be arranged. Or there are hundreds of cases to be studied, lists and tables prepared, field investigations made. He knows suspense, while experiments are running their course, frustration and disappointment when his approach to a problem has failed and he has nothing to show for months of earnest endeavour.

Rarely, happy chance presents him with significant information that he has not sought. Or he becomes so intent on the search that he finds something that is not there. In 1900, John Simon[2] wrote that mare's nests in science were tending to be a constantly diminishing quantity. But surely he under-estimated the enterprise of succeeding generations. Keen researchers often accumulate a nice collection which they can display for the amusement and edification of their friends. Fortunate are they who recognize the error before publication.

Matthew Arnold might have been describing the uncertainty and uneven course of an investigation :

> We cannot kindle when we will
> The fire which in the heart resides,
> The spirit bloweth and is still,
> In mystery our soul abides ;
> But tasks in hours of insight will'd
> Can be through hours of gloom fulfill'd.

One would qualify the last line. The path from inspiration to consummation may be arduous, but rarely gloomy ; it is brightened by visions of the goal.

[1] " As I Remember Him ", Little, Brown & Co., Boston, 1940, 101, 218, 292, 314.
[2] *J. Hyg. (Camb.)*, 1901, **1**, 3.

While inspiration may not be invoked at will, its advent can be encouraged by devoting time to meditation. The necessity of this is stressed by M. Arthus[3]:

> It is not in the turmoil of social life, not through academic chats nor laboratory gossip that we come to see the light, that interpretations become clear, that experiments are conceived, and conclusions reached. It is through solitary, profound, and sustained meditation. In order to make some progress in the experimental sciences one must meditate a great deal.

Yet meditation is likely to be sterile unless it has data to nourish the thoughts on, and the collection and analysis of data require effort. One who knew personally three Nobel laureates states that they were all extremely hard workers. Research is exacting also in time. An engrossing problem cannot be dismissed from the mind at 5 p.m. and is liable to intrude itself at midnight. Experimental animals may sicken or need observation at the most inconvenient hours. In their classical studies on the Wassermann test in general paralysis, Froude Flashman and Graham Butler[4] spent the first day collecting sera. In the evening they settled down to prepare reagents—there was no commercial supply in 1909—and that occupied the whole night. Next day they performed the tests, and in the afternoon, after 32 hours continuously without sleep, obtained their results.

Hard labour is inescapable, but its effectiveness can be increased by careful planning. Zinsser[1] relates that:

> Nicolle did relatively few and simple experiments. But every time he did one, it was the result of long hours of intellectual incubation during which all possible variants had been considered and were allowed for in the final tests. Then he went straight to the point, without wasted motion. That was the method of Pasteur, as it has been of all the really great men of our calling, whose simple, conclusive experiments are a joy to those able to appreciate them.

The first steps in planning are selecting the problem and defining the question to be answered. Many factors influence the choice, but the special opportunities offered by diseases of high local incidence should not be overlooked. Noteworthy advances of world-wide applicability have, for instance, attended the recognition of a goitre problem in New Zealand, its correlation with deficiency of iodine in soil, the detection of goitrogens in foods, and successful control with iodized salt.

The selected problem will be attacked with such skills and abilities as are at the researcher's command. Glenn Gardiner[5] suspects that investigation (of allergic problems) " might well require the curiosity of a Sherlock Holmes, the ingenuity of a Thomas Edison, the patience of a Diogenes, and the optimism of an idiot ". Talent is likely to make its mark; but the less endowed also have their triumphs, and the humblest can take heart with John Masefield's Highworth Ridden:

> I have seen flowers come in stony places;
> And kindness done by men with ugly faces;
> And the gold cup won by the worst horse at the races;
> So I trust, too.

Three centuries ago, René Descartes[6] urged those who study medicine to publish their results.

> I judge there was no better provision against a short life or lack of experience than faithfully to communicate to the public the little which I should myself have discovered, and to beg all well inclined persons to proceed further and then to communicate to the public all the things which they might

[3] Quoted by C. P. Richter, *Science*, 1953, **118**, 91.
[4] *Brit. med. J.*, 1909, **2**, 1019; and personal communication.
[5] *Ann. Allergy*, 1952, **10**, 732.
[6] Quoted by J. R. Schenken, *Science*, 1955, **121**, 184.

discover in order that the last should commence where the preceding had left off, and thus by joining together the lives and labours of many, we should collectively proceed much further than anyone in particular could succeed in doing.

Publication, while morally imperative, is not achieved without discipline. The accuracy of all statements must be verified, the irrelevant sorted out and discarded. The steps need arranging into a logical sequence, for rarely will the order of observations be the optimum for presentation. Edgar King[7] laments:

> There is only one form of labour which the average man shuns more instinctively and more consistently than the effort of thinking and of original observation; this is the task of carefully recording his observations and thoughts.

When the relevant facts have been set out and the immediate conclusions expressed, there comes the need for a second round of thinking. It is well to forget, as far as possible, the labour of the ascent, to make a fresh survey from the new vantage point, and to apply Macfarlane Burnet's rule[8]:

> No matter how immediate and practical the problem that is being studied, look at its significance from the point of view of each of those wider levels of medicine and biology for which it is inevitably significant.

This further consideration can greatly enhance the impact of the paper. And he who finally wins through to authorship earns, among other rewards, the commendation of Francis Drake:

> There must be a begynning of any great matter, but the continewing unto the end untyll it be thoroughly finished yeldes the true glory.

E. H. DERRICK.

[7] *Med. J. Aust.*, 1958, **2**, 853.
[8] *Med. J. Aust.*, 1953, **2**, 841.

HEREDITY IN PRIMARY GOUT[1]

B. T. EMMERSON[2]

From the Medical Professorial Unit, University of Queensland, Brisbane Hospital, Queensland

SUMMARY

Two family pedigrees are presented which contain persons homozygous for the gouty trait. In the first family, asymptomatic hyperuricæmia occurred in both parents of a patient with gout of extreme severity and rapid progression. The only relative with clinical gout was a cousin of the patient's mother. In the second family, gout or hyperuricæmia could be traced through five generations. From the union of one of the gouty males of this family and a hyperuricæmic female four hyperuricæmic and gouty children resulted. Three of these children are also hypertensive, and two have evidence of renal impairment. The patient in the index case in the first family had renal disease due to gout itself, while the second family demonstrates that renal disease in gouty subjects may also be due to the association in one family of hyperuricæmia and primary hypertensive vascular disease. Homozygosity for the gouty trait is suggested as a possible cause of gout occurring with undue severity or with an unusually wide family incidence.

SINCE the second century, gout has been recognized as being an hereditary condition (Galen, A.D. 138–201; "*Opera omnia*", *Editionem curavit* Carol Gottlob, Kuhn—Tomi 1–20, 1821–1823, Leipzig; quoted by Smyth, 1957). This aspect has recently been reviewed by Smyth (1957). Individual families with a high incidence of gout have at times been described (Scudamore, 1819), but an overall view suggests that clinical gout occurs in about 5% of the relatives of gouty patients. Folin and Lyman (1913) first recorded hyperuricæmia in an asymptomatic relative of a patient with gout, but the frequency of such an association was not realized until the more extensive study by Talbott (1940). He found that 25% of 136 relatives of 27 patients with gout had asymptomatic hyperuricæmia, and this finding led to further investigation into the inheritance of gout, serum urate levels of blood relations being used as the criterion of whether or not they were carriers of the gouty diathesis. A number of American investigators, particularly Smyth *et alii* (1948) and Stecher *et alii* (1949), have carried out large surveys, and generally concluded that the inheritance was not sex-linked, but was governed by a dominant autosomal gene which had varying penetrance. On the other hand, Hauge and Harvald (1955), as the result of an extensive survey in Denmark, concluded that inheritance was controlled by several genes (polygenic), and that the serum urate level was a "graded trait", similar to other bodily characters.

However, these and similar studies have thrown little light on the reason for the varied severity of gout. Some patients with gout may not develop symptoms until middle age, and then experience only mild, infrequent attacks. On the other hand, there is a group of patients whose first symptoms of gout develop soon after puberty, and in whom the condition progresses rapidly, with recurrent acute attacks, to death from renal failure before middle age. The following two pedigrees illustrate some factors which may determine the varying severity with which the gouty trait is manifest.

PEDIGREE I (Figure I)

Index Case

A stockman, aged 18 years, developed acute arthritis in the hands, which was rapidly followed by tophus formation in the fingers. Remissions were few and short, and progression was rapid towards limitation of movement of the fingers and wrists, partly by ankylosis, and partly by the mechanical effect of the urate deposits. Rather atypically, the first attack of acute arthritis in the lower limbs did not occur until three years after involvement of the upper limbs. Two unusual findings in this patient illustrate the severity of his gout. Firstly, on microscopic examination of the centrifuged urine deposit, acicular urate crystals were demonstrated (Figure II), suggesting tophus formation within the kidney. Secondly, progress radiographs showed extremely rapid development of destructive bone lesions (Figures III and IV). X-ray films of the lumbar part of the spine showed arthritic lesions at the apophyseal joints on the right, with associated new bone formation (Figure V); at autopsy, extensive chalky urate deposits were found corresponding with the region of radiological abnormality.

[1] Received on January 8, 1960.

[2] Senior Lecturer in Materia Medica and Therapeutics.

The patient's arterial blood pressure rose from 140/100 mm. Hg at the age of 21 years to 180/130 mm. Hg at the age of 25 years, when arterio-venous nipping could be seen in the optic fundus. The amount of protein in the urine averaged one gramme per day, and a urine concentration and dilution test (Fishberg, 1954) produced a range of specific gravities from 1·010 to 1·002. The average daily urinary urate excretion on a purine-free diet was 830 mg., and renal function tests revealed a urate clearance of 7·4 ml. per minute, a urea clearance of 36 ml. per minute, and a creatinine clearance of 67 ml. per minute. This renal impairment may have been a factor in the failure of uricosuric drugs to lower significantly the patient's abnormally high plasma urate level. At autopsy, evidence of gouty nephrosis (Sokoloff, 1957) was present, with macroscopic accumulations of chalky material and microcrystalline urate deposits both within the tubules and within the renal parenchyma. There was evidence of secondary pyelonephritis.

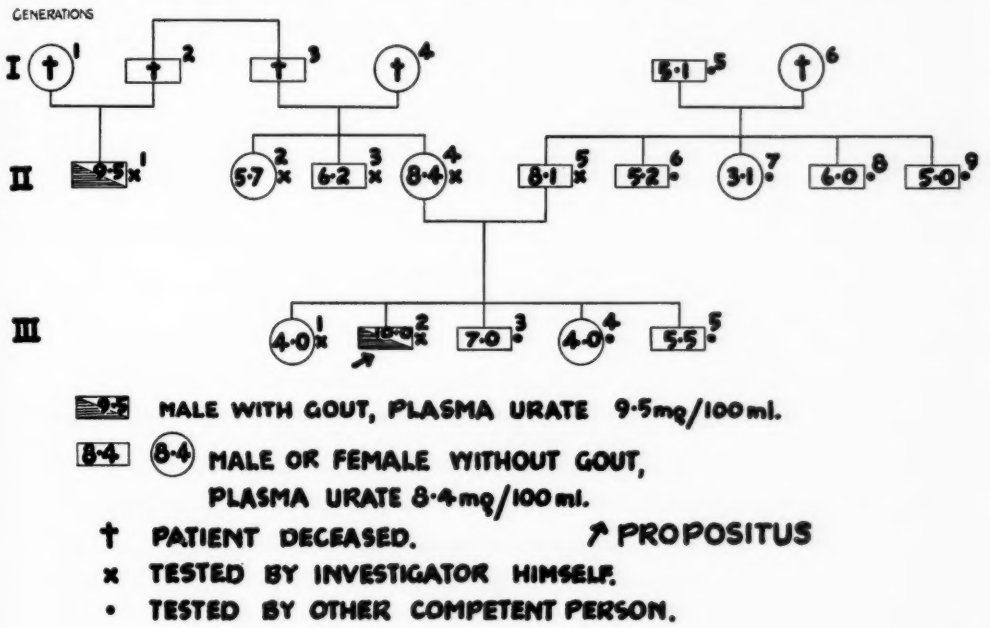

FIGURE I
Pedigree chart I

History of Other Members of Pedigree I

The index case appears on the first pedigree chart as "Generation III, No. 2".

This patient's father (Generation II, No. 5) was in good health at the age of 51 years, had a blood pressure of 140/80 mm. Hg, and denied any joint symptoms. Radiographs of the father's hands were normal. His serum urate level (King, 1951) was 8·8 mg. per 100 ml., and when the estimation was repeated on two occasions by the carbonate method of Henry *et alii* (1957), the levels were respectively 7·0 and 8·1 mg. per 100 ml.

The mother (Generation II, No. 4) was aged 50 years, and had a blood pressure of 150/85 mm. Hg. She had first been troubled by pains in her hands at the age of 46 years, but with reduction of her meat intake had been subsequently free of symptoms for twelve months. Examination of her hands revealed Heberden's nodes, and suggested osteoarthritis. X-ray films confirmed the presence of osteoarthritis of the interphalangeal joints. Small marginal defects were present, some of which appeared to be punched out, but these were such as could have been due as well to osteoarthritis as to gout. Her serum urate level (King, 1951) was 7·3 mg. per 100 ml. When the estimation was repeated by the carbonate method mentioned above some six and eight months later, and after the occurrence of her menopause, levels of 7·3 and 8·4 mg. per 100 ml. respectively were obtained.

The patient had four siblings. Of these, the two females (III, 1 and 4) had normal plasma urate levels. Of the brothers, the elder (III, 3) at the age of 17 years had a plasma urate level of 7·0 mg. per 100 ml., which is probably elevated for his age. The younger brother (III, 5) was prepubertal, and had a plasma urate level of 5·5 mg. per 100 ml. None of the siblings had any joint symptom.

Only two of the mother's siblings could be interviewed. One, a sister (II, 2), aged 57 years, had a plasma urate level of 5·7 mg. per 100 ml.; the other, a brother (II, 3), aged 52 years, had a plasma urate level of 6·2 mg. per 100 ml. Both of these fell within the "high normal" range, but were not definitely hyperuricæmic.

The plasma urate level of three of the father's brothers (II, 6, 8 and 9) and one of his sisters (II, 7) was estimated, their ages ranging from 48 to 35 years. The highest figure was in one brother (II, 8), whose plasma urate level was 6·0 mg. per 100 ml. The

patient's grandfather, aged 74 years (I, 5), also had a normal plasma urate level (5·1 mg. per 100 ml.).

The only relative of the patient found to have clinical gout was a maternal cousin (II, 1), aged 52 years, who had had recurrent attacks since the age of 40 years. The urate content of a single specimen of his plasma was significantly elevated—9·5 mg. per 100 ml.

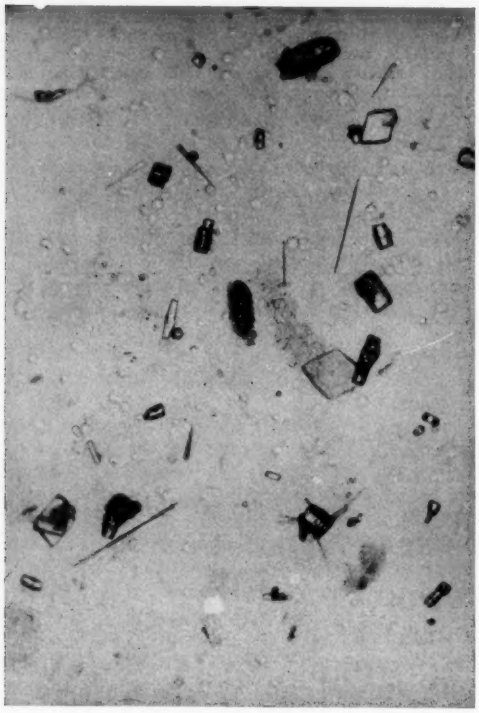

FIGURE II

Photomicrograph of centrifuged deposit of the urine of the patient in the index case of Pedigree I at the age of 23 years, showing several acicular urate crystals

This family pedigree, then, shows a patient (III, 2) with severe primary gout and secondary renal disease, in whom hyperuricæmia was present on both paternal (II, 5) and maternal (II, 4) lines of descent. Overt gout was otherwise present only in a cousin of the patient's mother (II, 1).

PEDIGREE II (Figure VI)

Index Case

A male school-teacher first developed podagra at the age of 25 years. Typical attacks of gouty arthritis, each relieved by colchicine, recurred with increasing frequency during the following eight years, but no cutaneous tophi developed. Between attacks, remission was complete. At the age of 33 years the arterial blood pressure was 140/100 mm. Hg, and a moderate degree of proteinuria was found. A urine concentration and dilution test (Fishberg, 1954) revealed a range of specific gravities from 1·016 to 1·004. The average daily urinary urate excretion on a purine-free diet was 350 mg., and clearance measurements, made over a 24-hour period while the patient was taking this diet, revealed a urate clearance of 4 ml. per minute, a urea clearance of 34 ml. per minute, and a creatinine clearance of 40 ml. per minute. Treatment with probenecid increased urinary urate excretion, and diminished the frequency of his attacks of gout.

History of Other Members of Pedigree II

The index case appears on the second pedigree chart as "Generation IV, No. 5".

This patient's father (III, 3) and two of his uncles (III, 1 and 2) had been similarly affected with gout, the father having died at the age of 52 years of a "coronary occlusion". His paternal grandfather (II, 1) and great-grandfather (I, 1) had also suffered from gout. The patient's mother (III, 4), aged 65 years, was hypertensive (blood pressure 240/140 mm. Hg), and had suffered several "little strokes". Her plasma urate level (Henry *et alii*, 1957) was 6·9 mg. per 100 ml., the blood urea content being normal (see Table I). The history and physical examination did not suggest clinical gout.

Two of the patient's brothers suffered from gout. The elder brother (IV, 2), aged 44 years, had first developed a painful joint four years previously. His

TABLE I

Details of Members of Pedigree II

Generation and Number (Figure VI)	Age (Years)	Blood Pressure (Millimetres of Mercury)	Protein in Urine (Grammes per Litre)	Urinary Urate Level (Milligrammes per 24 Hours)	Plasma Urate Level (Milligrammes per 100 ml.)	Blood Urea Level (Milligrammes per 100 ml.)	Plasma Creatinine Level (Milligrammes per 100 ml.)
III, 4	65	240/140	Nil	—	6·9	26	—
IV, 2	44	150/100	Nil	960	9·6	20	1·3
IV, 3	42	200/135	1·3	370	9·2	60	2·4
IV, 4	39	130/90	Nil	—	4·9[1]	15[2]	—
IV, 5	33	140/100	Heavy cloud	350	8·2	40	1·5
V, 2	13	115/70	Nil	680	7·2	36	0·9

[1] Blood uric acid level, estimated by Folin's cyanide method; normal for this laboratory, 1·6 to 3·5 mg. per 100 ml.
[2] Blood urea nitrogen level; normal for this laboratory, 7 to 20 mg. per 100 ml.

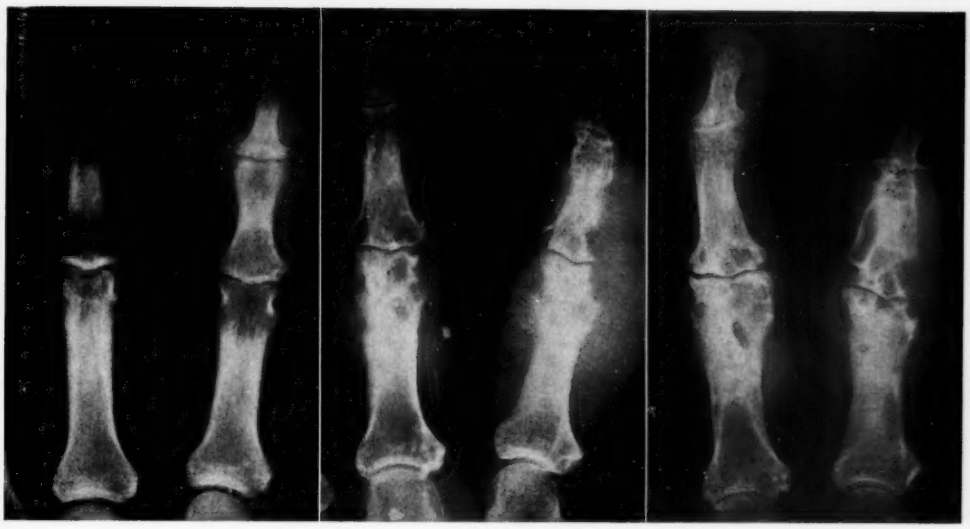

FIGURE III

Radiographs of the fingers of the patient in the index case of Pedigree I, to show progress changes from 1954 to 1956 and 1958. Surgical drainage of tophi had been undertaken in 1957

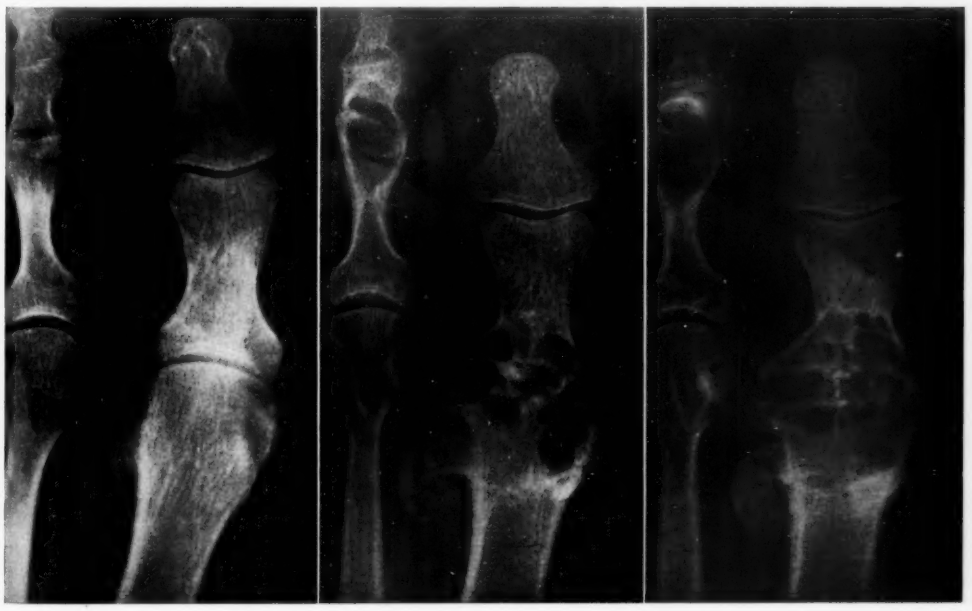

FIGURE IV

Radiographs of the toes of the patient in the index case of Pedigree I, to show progress changes from 1954 to 1957 and 1959

attacks of gouty arthritis, though severe and frequent, had each been of short duration (approximately 24 hours), and had been rapidly relieved either with colchicine or with aspirin. They had involved the knee, ankle or wrist joints, but there was no clinical or radiological evidence of tophus formation. Rubbing over the capsule of any of these joints would readily produce local soreness. This feature, together with the lack of involvement of the metatarso-phalangeal joint of the great toe and the brevity of the attacks, made his gout somewhat atypical. His blood pressure was 150/100 mm. Hg, and there was no proteinuria

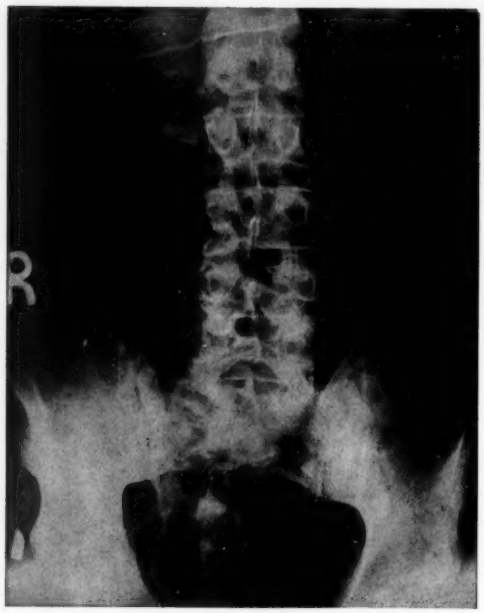

FIGURE V

Radiographs of the lumbar part of the spine of the patient in the index case of Pedigree I at the age of 26 years, showing arthritic lesions of the lower apophyseal joints on the right side with new bone formation as indicated by the arrows

(see Table I). The plasma urate level was 9·6 mg. per 100 ml. and the plasma urea level was normal. On a low purine diet, he excreted 960 mg. of urinary urate in 24 hours, the creatinine clearance being within the normal range. This brother's wife (IV, 1), and his daughter, aged 18 years (V, 1), had plasma urate levels within the normal range; but his only son (V, 2), at the age of 13 years and shortly after puberty, had a plasma urate level of 7·2 mg. per 100 ml. On a purine-free diet this son (V, 2) excreted 680 mg. of urate in a 24-hour urine specimen. He was in good health, apart from complaining of transient aching in the knees after exertion. The urinary urate levels in this father (IV, 2) and son (V, 2) were both elevated, suggesting that their hyperuricæmia may have been due to "over-production" of urate.

The other brother (IV, 4) lived in South Australia, and the following information was obtained from his private medical practitioner. His attacks of gouty arthritis had been typical (similar to those in the index case—IV, 5) and had first occurred at the age of 31 years; his blood pressure was 130/90 mm. Hg, and there was no proteinuria. The results of his blood tests are given in Table I; they reveal no evidence of renal failure.

The sister (IV, 3), on the other hand, had definite renal impairment and hypertension. She was aged 42 years, was premenopausal, and when first examined had suffered only one episode of joint pain two years earlier. This had involved the first metatarso-phalangeal joint and had followed trauma; but the severity of the pain had been disproportionate to the amount of the trauma. She has since suffered a spontaneous attack of acute podagra, which was characteristically relieved by colchicine. Her arterial blood pressure was consistently elevated (200/135 mm. Hg), and her urine contained 1·3 grammes of protein (Esbach) per litre. Though this proteinuria had been noted for some ten years, there was no definite history of primary renal disease. The plasma urate level was 9·2 mg. per 100 ml., and this elevated level seemed somewhat out of proportion to the degree of renal impairment (the plasma urea level was 60 mg. per 100 ml., and the plasma creatinine level was 2·4 mg. per 100 ml.—see Table I). Renal function tests showed a urate clearance of 5·2 ml. per minute, a urea clearance of 21 ml. per minute, and a creatinine clearance of 39 ml. per minute. While the patient was on a purine-free diet, the daily urinary excretion of urate was 370 mg. Her daughter (V, 3), aged 23 years, had a normal plasma urate level.

This family pedigree traces gout or hyperuricæmia through five generations. It records four siblings with gout (IV, 2, 3, 4 and 5), children of hyperuricæmic parents with vascular disease (III, 3 and 4). These four siblings were hyperuricæmic, three were hypertensive and two showed evidence of renal impairment.

THE SIGNIFICANCE OF THE PLASMA URATE LEVEL

When Jacobson (1938) showed that urate could be more reliably estimated in serum or plasma than in whole blood, he pointed out that 97% of a group of non-gouty patients had a serum urate level below 6 mg. per 100 ml. This figure was then accepted as the critical value upon which later investigators, especially Talbott (1940), Smyth and Freyberg (1942) and Stetcher and Hersh (1945), classified relatives as being normal or hyperuricæmic. Later, Smyth *et alii* (1948) made allowance for the variation of serum urate levels with sex, as had been previously pointed out by Brøchner-Mortensen (1937). In their extensive survey of gouty families, they accepted as hyperuricæmic males with serum urate levels above 6 mg. per 100 ml. and females with urate levels above 5 mg. per 100 ml. Stecher *et alii* (1949), while allowing for the difference with sex, found a "clear cut division between hyperuricæmic and normal" at a serum level

of 6·5 mg. per 100 ml. Hauge and Harvald (1955), on the other hand, have found it impossible to draw a line between normal and pathological serum urate levels, but found that the serum urate values for siblings of gouty patients significantly exceeded those in a control group. Hendry *et alii* (1959) also found that a histog am of the serum urate levels of a group of 400 blood donors approximated to a normal distribution curve rather than to a bimodal one. Excluding the upper

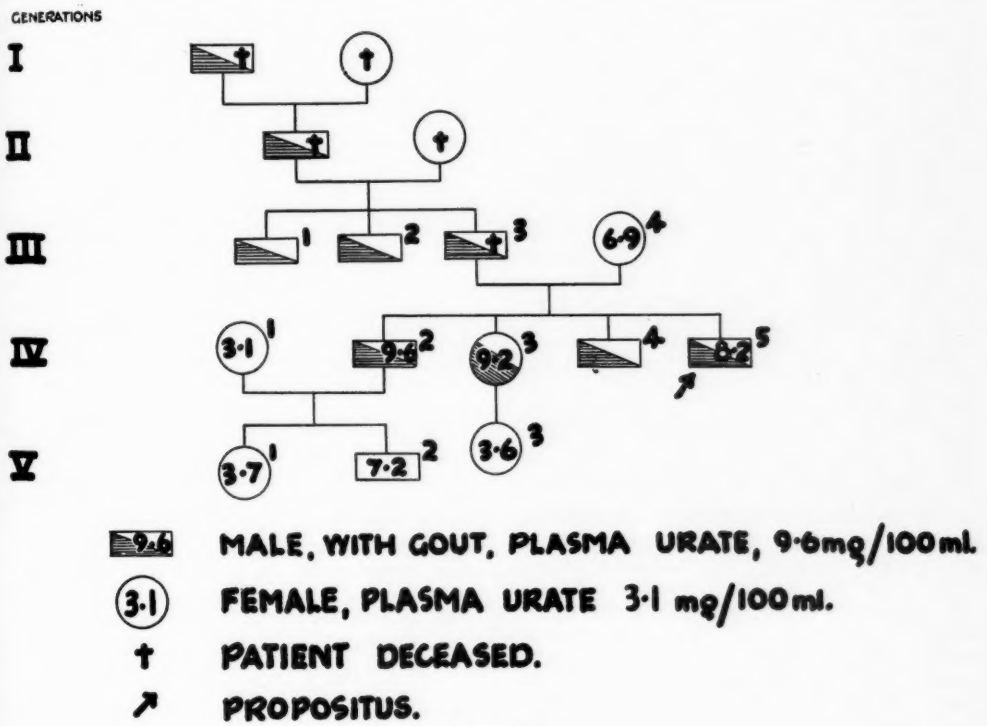

FIGURE VI
Pedigree chart II

and lower 10% of their results as being suspiciously abnormal, they found the resulting range for normal males to be 4·1 to 7·0 mg. per 100 ml. and for normal females 3·1 to 6·0 mg. per 100 ml.

Another factor to be considered in determining the upper limit of the normal serum urate level is the biochemical method used. Most estimations before 1949 were done by modifications of the Folin method (Folin, 1933). The Danish estimations were done by a uricase method (Praetorius, 1949), and those of Hendry *et alii* (1959) by the carbonate method of Caraway (1955). This last mentioned author, using a North American population, gives the normal range as 3·0 to 5·5 mg. per 100 ml., making no mention of the accepted sex difference. In the present investigation, the figures marked " · " were obtained by this method in the Commonwealth Health Laboratory at Toowoomba, whereas those marked " × " were carried out under personal supervision by the modified carbonate-phosphotungstate method of Henry *et alii* (1957). Plasma urate estimations carried out by this method on 200 normal or hospitalized members of the local populations, in whom there was no evidence of any gouty trait, have confirmed the normal range established by Hendry *et alii* in Newcastle (Australia). In this study, therefore, 7·0 mg. per 100 ml. for males, and 6·0 mg. per 100 ml. for females, have been taken as the levels above which relatives are regarded as being hyperuricæmic. The acceptance of lower levels would classify a correspondingly greater proportion of relatives into the hyperuricæmic category.

DISCUSSION

One of the earliest reports in which both parents were affected by either gout or hyperuricæmia was that of Scudamore (1819). He reported one such family, in which both parents and all four children, both male and female, were afflicted with gout. Smyth and Freyberg (1942) reported a family in which the father was gouty and the mother hyperuricæmic, and of the four children, the three sons had gout and the daughter, aged 35 years, was hyperuricæmic. This family was again discussed, together with 18 other families, by Smyth *et alii* (1948). These authors also mention another family in which the parents, similarly affected, had two hyperuricæmic sons, aged 23 and 22 years. They suggested that gouty patients with two hyperuricæmic parents might be more severely affected than those with only one hyperuricæmic parent, and that such patients might have earlier ages of onset of acute attacks. They demonstrated the latter hypothesis to be true in the first family mentioned, but were not able to demonstrate the increased severity of gout in such homozygous patients.

Stecher *et alii* (1949) also reported one family in which the father was gouty and the mother hyperuricæmic. There were four daughters of this union, all aged under 28 years, with normal serum urate levels at the time of publication. This fact is probably of limited significance, in view of the tendency for hyperuricæmia to become manifest in females only after the menopause. These authors also hold that when symptoms develop early in life, the disease is apt to be severe and progress rapidly. A similar family is recorded by Gutman and Yü (1952). Of 10 children of a union of two people with asymptomatic hyperuricæmia, four had the same condition and two others had frank gout. These authors accepted the conclusions of the other groups mentioned, that hyperuricæmia was inherited as a dominant autosomal character with incomplete penetrance.

Hauge and Harvald (1955), investigating heredity in gout and hyperuricæmia in Denmark, also found serum urate levels to be significantly higher in the siblings of gouty patients than in a control group. In contradistinction to the American workers (Smyth *et alii*, 1948; Stecher *et alii*, 1949), they found that the distribution curve of the serum urate levels of the patients' siblings was not significantly different from a normal curve, and that they did not fall into hyperuricæmic and normal groups. Their findings suggested a polygenic inheritance for hyperuricæmia, similar to that found in the inheritance of other normal bodily characters, such as height.

Which of these two theories better explains the hereditary nature of gout is not yet certain; but with either it would be expected that the occurrence of an elevated serum urate level in both parents would be associated with an increased tendency to hyperuricæmia in the children. If the inheritance was by a dominant gene, children with two hyperuricæmic parents could be homozygous for this trait, and might tend to develop gout at an earlier age and with greater severity than others. If the inheritance was polygenic, a similar state could still be expected in the children of two adults with elevated serum urate levels.

In the first case reported here, gout began at an unusually early age (18 years), was of extreme severity, and was associated with extensive and rapid urate deposition and the early development of renal damage and hypertension. Examination of the patient's parents revealed them both to be hyperuricæmic, without definite gout and without evidence of hypertension or renal disease. This incidence of hyperuricæmia in the patient's antecedents may explain the unusual severity of this patient's gout.

The second family mentioned here is somewhat more complicated, in that while the father was gouty and the mother hyperuricæmic, the father died of a myocardial infarct and the mother was severely hypertensive. Her hypertension was regarded as being primary, there being no clinical gout which might have produced a gouty nephropathy, and the blood urea content being normal. The family history, then, indicates arterial disease and hypertension, as well as hyperuricæmia, on both sides of the family. Both of these may have secondary effects on the kidneys. All four children were hyperuricæmic and had suffered attacks of gout, and three had varying grades of hypertension. Two had proteinuria and renal impairment, the daughter (who had the higher blood pressure) having the greater renal impairment. One, at least, who had no renal impairment, excreted excessive amounts of urate, and the son of this man, at the age of 13 years, was hyperuricæmic. It is suggested that these siblings may be hyperuricæmic because both parents possessed this trait, and that further cardio-vascular disease has been superimposed in varying degrees because of the family tendency (Fraser Roberts, 1957). In this family gout and hyperuricæmia have been traced through five generations.

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REPORT OF A FAMILY SHOWING "MIRROR" MOVEMENTS[1]

CHARLES CRAWFORD[2]
From Napier Hospital, New Zealand

SUMMARY

A family is described showing mirror movements in five members of two generations, and a new hypothesis is advanced which may help to throw light on the cause of the condition.

THE inability to dissociate the movements of the two arms results in the phenomenon known as "mirror" movement. A patient with this disability who performs an action with one hand finds his other hand moving involuntarily in a symmetrical fashion. He extends his right index finger to point, and his left index finger does the same; he grasps an object with his right hand, and his left hand uselessly performs a similar movement as if grasping a similar invisible object. The term mirror movement was first used by Erlenmeyer in 1879 in the description of similar cases, and is employed because the two hands, moving symmetrically, give the impression of one hand's movements being the mirror-image of the other's.

This rare condition is usually associated with other gross anomalies, the most common being those of the atlanto-occipital region and cervical part of the spine, as seen in the Klippel-Feil syndrome (Bauman, 1932). It has also been described in patients with minor degrees of damage to the pyramidal system after birth injuries (Berlin, 1951), and also in a boy mentally retarded from phenylpyruvic oligophrenia (Friedman and Levinson, 1954). It has much more rarely been described in otherwise normal children, the only two reports I have been able to trace being those of Drinkwater (1913), who described the condition through four generations of a family, and Smith (1947), who described it in a father and son.

The present report describes the condition in five members of two generations of a family, and is submitted because of its rarity, and also to suggest a new hypothesis as to its ætiology.

CASE REPORT

The family first came to my notice when the mother brought her child, aged eight years, because she was clumsy in her school-work, and her school-teacher had wondered if she was a "spastic". The mother knew the condition well, as three of her four children were affected, as well as her husband and his sister (see family tree, Figure I). Only the one child will be described in detail, since she is typical, the other members of the family differing only in the degree to which they are affected.

Y.W., aged eight and a half years, was born by a straightforward labour after a normal pregnancy. The mother, who was on the lookout for this condition, considers that she first noticed it at the age of about three months, when the child was beginning to develop finger movements in addition to the coarser limb movements that are the main activities of a younger infant. The child developed normally, except that she made no attempt to walk till the age of 22 months, and then quickly learned to do so. She fed herself and

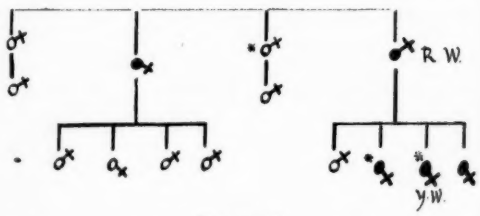

FIGURE I

Family tree. Solid circles, members of the family with mirror movements; open circles, unaffected members; asterisks, members with preauricular sinus

was able to dress herself at a normal age, and at school learned to write right-handedly as neatly as other children, although she writes more slowly and deliberately. She is in an average school class for her age, but has great difficulty in learning the piano, which, of course requires complete dissociation of hand movements. Her older sister, aged 11 years, who is also affected, is now playing two-handed pieces, and is up to average, according to the mother.

On examination of the patient, she was found to be a bright intelligent child, whose only demonstrable abnormality apart from the mirror movements was a preauricular sinus, visible as a pinhead sinus plugged with sebum between the helix and the tragus of the right ear. No other congenital abnormalities were found, and in particular she showed no abnormality in the pyramidal system.

The mirror movements were obvious, being most noticeable in the hands and fingers, less so in the wrist and forearm rotators, and visible at shoulder-level only as a slight flickering of deltoid fibres during abduction

[1] Received on February 24, 1960
[2] Assistant Physician.

of the other arm. If she picked up a penny from the table with one hand, the other hand would perform identical movements, as if picking an invisible penny from the air; and if she squeezed the examiner's finger, the fingers of the other hand would also close. Similarly, if she was asked to point the index finger of one hand, both hands would move, and frequently the wrong hand would move first. This wrong choice of hands occurred even if the examiner pointed to the required hand, or touched it.

With concentration the child could partially suppress the mirror movements. For instance, if she was given two pennies to hold, one in either hand, and then told to drop only one, she could with difficulty manage to keep the fingers of the other hand closed round its penny. However, the effort of suppression overflowed into the active hand, so that the desired movement of opening the hand became stilted and lacked the normal free flow. The hand moved in a stiff fashion with antagonists contracting as well as synergists, producing a very similar appearance to the hand-opening efforts of a patient with myotonia.

When both arms were used together she was as agile as other children; for instance, she could perform well with both arms together the rapid alternating pronation-supination movements of the forearm used in testing cerebellar function, but with either arm separately she became clumsy, partial suppression obviously stilting the arm movement.

Outside the arms there were no abnormal associated movements. Both legs moved independently, and the toes on either foot could be moved without a flicker of movement in the other foot.

The face is a part of the body in which symmetrical reflex movements normally occur, as on blinking, smiling and talking, and it was expected that the patient might well have difficulty in dissociating the movements of either side of her face, but this was not so. She could show her teeth on each side independently, and she could put out her tongue to either side at will. She could also wink with one eye, not with adult precision, but as well as any eight-year-old can, with only slight contraction of the opposite orbicularis oculi.

In spite of this gross abnormality of movement, no sensory changes were found, and in particular there was no difficulty in knowing which side of the body was being stimulated, or in distinguishing simultaneous bilateral stimuli from unilateral ones; the difficulty in moving the correct hand first, mentioned above, was the result of a motor, not a sensory defect.

Her writing was average for her age in neatness, although she wrote in an awkward attitude, and the left hand, which held the paper, moved in partly suppressed mirror image of the right. Her spelling and vocabulary appeared average for her age.

In view of the possible connexion of this condition with mirror-writing, it is noted that the child had great difficulty in mirror writing with her left hand, either on its own, or simultaneously with right-handed writing. (Mirror writing, of course, goes from right to left on the page, with the words and letters reversed, so that when viewed in a mirror the image of the writing becomes identical with normal writing. Leonardo da Vinci habitually used mirror writing, and a normal person can, with concentration, mirror-write with his left hand, particularly if he simultaneously writes the same words normally with his right hand on another piece of paper.)

Of the other available affected members of the family, R.W., aged 51 years, father of the child under consideration, was the least obviously so, because he had learned to inhibit the mirror movements to the extent that the only visible sign was a flickering contraction of the tendons of the dorsum of the passive hand when the other hand was used. One affected sister, aged 10 years, is becoming left-handed, but the rest of the family are right-handed. There are no stammerers. The condition produces very little disability in this family, although the affected aunt found it impossible to learn the pianò; but the children are slowly learning to play normally. The father claims that, apart from being a social embarrassment, the condition is perhaps an advantage to him, in that he is ambidextrous with most tools. The only associated abnormality in this family is the preauricular sinus already mentioned. Two of the affected sisters have it (one bilaterally), and an uncle not suffering from mirror movements also has one. No earlier generations of the family are known to be affected. Radiographic examination of the cervical part of the spine and the atlanto-occipital region showed no abnormality.

DISCUSSION

Compared with other affected families, this family has the condition only in a mild form. The family described by Smith (1947) had a marked disability, in that the father was unable to climb a ladder, for whenever he reached towards the next rung, both hands would let go at once; and he could not open the barn door without first putting down the milk bucket from the other hand, otherwise he dropped the bucket. The son, 12 months old at the time of the report, had a similar inability to climb up the sides of his play-pen.

Apart from Smith's family, and that described by Drinkwater (1913), the remainder of the subjects described all showed other gross developmental defects.

Bauman (1932) described six cases of Klippel-Feil syndrome, in four of which the abnormality of the cervical part of the spine was associated with mirror movements. One patient out of the four was mentally retarded. An aunt and a cousin of one patient had mirror movements and were mentally retarded, but neither had any noticeable abnormality of the cervical part of the spine.

Berlin (1951) described two cases of mirror movements, both patients having signs of pyramidal damage, probably from birth injury, and believed that all patients showing mirror movements also showed signs of pyramidal tract damage, such as hyperreflexia and Babinski signs. This assumption is not true of the family described in the present report.

Friedman and Levinson (1954) described a boy, mentally retarded as the result of phenyl-pyruvic oligophrenia, in whom mirror movements were not noticed till he was five years old. They look on mirror movements as a specialized form of associated or synkinetic

movement, and quote Monrad-Krohn's classification of synkinetic movements into the following three types:

(1) Generalized synkinetic movements, consisting of involuntary movements in an extremity tending to produce the typical attitude of predilection of the extremity. An example of such a movement is the involuntary extension of all joints in a lower extremity paralysed by pyramidal tract damage when the non-paralysed side is voluntarily exerted.

(2) Co-ordinated synkinetic movements, consisting of involuntary movements of synergic muscle groups accompanying a voluntary movement in the same limb; for example, the Strumpell phenomenon, which consists of an involuntary dorsiflexion and supination of the foot occurring on voluntary flexion of the knee joint in the same paretic limb.

(3) Symmetrical synkinetic movements, consisting of involuntary movements of imitation in one extremity accompanying voluntary movements of the other side. An example of this is the involuntary flexion movements of the fingers of a paralysed hand when a patient squeezes with the normal hand.

They suggest that mirror movements come under this third heading, and that they are associated movements of the symmetrical, imitative type. They also point out that in infants there is a tendency for movements of one limb to be accompanied by a similar involuntary movement of the opposite limb; but that this disappears when they acquire normal coordination and muscle power.

The cause of mirror movements is quite obscure. Ford (1952) quotes two hypotheses: first, that both pyramidal tracts arise from one side of the cerebral cortex, so that identical stimuli are sent to both sides of the body; second, that there is a partial failure of decussation of the pyramidal tracts in the medulla. Impulses then meant for only one side of the spinal cord actually overflow to both sides. Bauman, on the other hand, suggests that the defect is at the level of the corpus striatum.

None of these hypotheses explains the observed facts: (i) that the mirror movements occur mainly in the skilled and highly educated actions of the hands; (ii) that they can usually be suppressed; (iii) that they may arise *de novo* in a previously normal person who acquires hemiplegia through cortical or other damage.

A further hypothesis is now advanced which depends on three well-known properties of cortical function.

(1) As one descends through the various levels in the hierarchy of control of movement, one meets laterality only at the level of the motor or pyramidal cortex. Below that level, stimulation of one pyramidal cortex or tract anywhere along its course produces a movement on one side only. However, above the level of the precentral gyrus motor activity of both sides is controlled by the posterior part of the left parietal cortex (in right-handed people), especially by the left supramarginal gyrus (Brain, 1955), so that at that level control of motor activity of both sides is from one side only. Higher again in the hierarchy of control, it is possible that the thoughts which precede the final movement are represented by diffuse cortical activity, having no "sidedness".

(2) Mirror movements occur normally in early life. A new-born infant moving one hand tends to move the other as well, and only later does he learn to use either hand independently. Not until much later, when he is several years old, does a child learn to close one eye without the other, as in winking, and as he learns, there is obvious difficulty in suppressing the movement of the other eye. Some movements most of us never learn to dissociate—raising one eyebrow, dilating one nostril and expanding only one side of the chest are examples.

(3) The learning of a skill by one hand—for instance, writing—is accompanied by the passive acquisition by the other hand of similar skill, albeit much less well developed. With concentration, most of us can produce mirror writing with the left hand, which, if read with the aid of a mirror, is at least recognizably our own writing, with our own personal idiosyncrasies. Thus, the same store of kinæsthetic memories appears to serve both hands, although it is more easily available to the dominant hand.

Thus, the probable chain of events in initiating a movement may be indicated as follows: initiating thought (probably diffusely located)→unilateral activity (in left supramarginal gyrus)→stimulation of both motor cortices→immediate suppression of one side, with resultant unilateral pyramidal activity.

The mode of action of this suppressive mechanism is unknown; but the crux of this paper lies in the suggestion of normal unilateral movement being the result of the suppression of one half of a bilateral cortical activity, and, as has been suggested in describing the efforts of a child to wink with one eye, this suppression has to be learned as a complex skill in itself. It may also be lost after cerebral damage, as is shown by many hemiplegics who, when asked to close their normal hand, may also partially close the paretic one.

It is suggested, therefore, that mirror movements are the result of a failure in the suppression

of bilateral pyramidal activity, the defect being above the level of the pyramidal cortex. The defect may be congenitally determined, as in the family reported here, and in Klippel-Feil syndrome. In the latter condition the defect is not due to the abnormality in the cervical part of the spine, but simply associated with it, the association being due to some common basic genetic fault. On the other hand, the defect may result from birth injury, when the pyramidal signs of hyperreflexia and Babinski response are simply signs of other cerebral damage, or from the various other causes of hemiplegia. Finally, it may be acquired later in life from chemical damage to the cortex such as occurs in phenylpyruvic oligophrenia.

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THE SEASONAL DISTRIBUTION OF HOSPITAL ADMISSIONS FOR ASTHMA IN BRISBANE[1]

E. H. DERRICK,[2] R. H. THATCHER[3] and L. G. TRAPPETT[4]
From the Queensland Institute of Medical Research, Brisbane

SUMMARY

The monthly distribution of admissions to the Brisbane Children's Hospital for asthma for the three years July, 1955, to June, 1958, showed a minor wave in the spring months of October and November, and a major wave in autumn and early winter culminating usually in May.

The monthly distribution of asthmatic bronchitis was almost identical with that of asthma. For a truer index of asthma admissions, the two conditions were combined.

The characteristic seasonal distribution of asthma was evident (*a*) in each sex, (*b*) in each year of age except the first, (*c*) in each of the three years of the study, (*d*) in different geographical sections of Brisbane City.

Among admissions of asthmatic children, about 10% belonged to the spring wave and 45% to the autumn wave; 45% were apparently non-seasonal.

The monthly distribution of admissions for infective (non-asthmatic) bronchitis to the Brisbane Children's Hospital over the same period was different. There was a single peak, in mid-winter. This seasonal difference shows that the spring and autumn waves of asthma cannot be ascribed to bronchial infection.

Analysis of admissions for asthma to the Brisbane Hospital for the same three years showed that the seasonal variation diminished with age. In asthmatics aged 12 to 49 years, a spring wave was barely discernible and the autumn wave was responsible for only about 20% of admissions. In those aged over 50 years, no consistent seasonal variation was evident.

Males predominated among asthmatics admitted to the Brisbane Children's Hospital, females among those admitted to the Brisbane Hospital.

Monthly correlations did not show any consistent relation between the seasonal distribution of asthma and variation in atmospheric pressure, temperature, humidity, rainfall, hours of sunshine, or speed or direction of the wind. Low wind speeds and westerly winds usually prevailed at the time of the autumn wave of asthma, but the association was probably incidental.

The most likely cause of the spring and autumn waves of asthma was a prevalence of air-borne pollens.

THE prevalence of asthma is a challenge to preventive medicine. It is a common illness and causes a great deal of distress to sufferers. The mortality, while low, is by no means negligible—about 80 deaths are ascribed to it annually in Queensland.

This paper records an analysis of admissions for asthma to the Brisbane Children's Hospital and the Brisbane Hospital for the three years July, 1955, to June, 1958.

In general, an asthmatic attack is readily recognized, and recorded diagnoses are reliable for analysis. One diagnostic difficulty which may arise—the distinction from bronchitis in infants—will require notice later. Cases of asthma in which admission to hospital is required are a small and selected fraction, tending to be severe in grade or associated with complications. When a patient presents with an asthmatic attack, he is usually treated first in the casualty room. Only if the attack persists or recurs in spite of treatment is he admitted. Others are referred to hospital because private treatment has failed. Admission would be favoured by remoteness of the patient's home from hospital, inadequacy of home care or inclement weather.

BRISBANE CHILDREN'S HOSPITAL CASES

Paterson and Moncrieff (1947) have pointed out the difficulty of distinguishing between asthma and bronchitis in infants, as the clinical features overlap. Bronchitis may lead to obstruction of air tubes from exudate, swelling of the wall and perhaps spasm; asthma is associated with catarrh. Attacks of asthma are

[1] Received on January 15, 1960.
[2] Deputy Director.
[3] Student Assistant.
[4] Student Assistant.

apt at first to be regarded as recurrent bronchitis until their frequency, their rapid onset and offset or the detection of a wheeze leads to the correct diagnosis. A compromise diagnosis of asthmatic bronchitis is frequently made, particularly in infants, but also in older children. Bronchopneumonia may complicate either asthma or bronchitis. The close relationship between these conditions is illustrated in the following clinical histories.

Alan, born in February, 1956, was admitted to hospital with "acute bronchitis" in July, "asthmatic bronchitis" in August and "bronchopneumonia" in September.

When Chris, aged 27 months, was examined in April, 1956, a history was given of bronchitic attacks over a period of 14 months. The diagnosis on this occasion was "asthmatic bronchitis". Eight months later, he was readmitted to hospital with "asthma".

Francis, a microcephalic, born in May, 1956, was in hospital seven times from March, 1957, to May, 1958. The successive diagnoses were "bronchopneumonia", "bronchitis and infantile eczema", "asthmatic bronchitis", "asthma", "asthma", "asthma", "bronchitis and subsiding asthma".

In the course of two years, Barbara, aged three years on the first occasion, was admitted to hospital four times with the diagnosis of "asthmatic bronchitis" and eight times with that of "asthma". "Asthmatic bronchitis" was applied mostly to the earlier episodes.

The overlapping of diagnoses made it desirable to study cases of asthmatic bronchitis and bronchitis as well as those of asthma. The diagnoses in the cases studied are shown in Table I. The comparatively few variants and complications were included for analysis in the three main groups. Admissions of asthmatic subjects for reasons other than acute attacks, or when asthma was not regarded as the primary cause of the illness, were not included.

The number of cases in each group in each year of the study is shown in Table II. The 556 admissions for asthma and asthmatic bronchitis represented 372 patients, as some were admitted repeatedly. Two boys were each admitted 13 times during the three years and one girl 12 times. Bronchitics were rarely readmitted with bronchitis, but 21 were in hospital on another occasion with asthmatic bronchitis or asthma. A pair of identical twins were in hospital together with asthma, and there were six other pairs of siblings. No asthmatic attack in this series ended fatally, but asthma probably contributed to the death in February, 1957, from bronchopneumonia, of a girl, aged two years.

Patients resided in all parts of the City of Brisbane as well as in its outskirts. Those who lived outside Brisbane have been excluded from the study in order that any conclusions may be referable to a clearly defined area. Brisbane is situated at 27·5° south latitude and 153° east longitude. The city boundaries extend for up to 15 miles from the centre and enclose an area of 385 square miles, of which about 105 are built up, the remainder being rural.

TABLE I

Diagnoses in Cases Studied, Brisbane Children's Hospital, 1955–1958

Diagnosis	Number of Cases
"Asthma" or "bronchial asthma"	294
"Asthma" and some lesion not implying inflammation in bronchi or lungs ("eczema", "tonsillitis", "otitis", "sinusitis", etc.)	26
Total: asthma group	320
"Asthmatic bronchitis"	191
"Asthma" and "bronchitis"	21
"Asthma" or "asthmatic bronchitis" and "bronchiolitis"	3
"Asthma" and "bronchiectasis"	3
"Asthma" or "asthmatic bronchitis" and "pneumonia"	18
Total: asthmatic bronchitis group, asthma with implied inflammation of bronchi or lung	236
"Bronchitis" or "acute bronchitis"	335
"Allergic bronchitis"	11
"Bronchiolitis"	22
"Bronchitis" and "pneumonia"	8
Total: bronchitis group	376

Of the subjects studied, males were more numerous than females (Table II). However, the percentage of males was similar to that—59%—for all admissions to the hospital for 1955–1958.

TABLE II

Annual Admissions and Sex for the Three Groups Defined in Table I

Group	Period			Total	Male Percentage
	1955–1956	1956–1957	1957–1958		
Asthma ..	115	98	107	320	59
Asthmatic bronchitis	71	93	72	236	65
Bronchitis ..	107	132	137	376	59

The age distribution of asthma was irregular, and varied much from year to year. Cases were well represented in each year in the age range of the hospital (Figure I). The youngest patients were three infants, aged four months. In view of the difficulty of diagnosis often experienced in infancy, it should be mentioned that the notes of some of the youngest infants record convincing accounts of asthmatic attacks. This was so, for instance, with Raymond, aged

five months, and the diagnosis was supported by readmissions for asthma when he was aged 20 and 21 months. The parents of a number of older asthmatic children stated that their attacks had begun in early infancy. The age distribution of bronchitis was quite different; this was mainly a disease of infancy, and the incidence fell rapidly with increasing age. As might be expected, the curve for asthmatic bronchitis lay mostly between the other two.

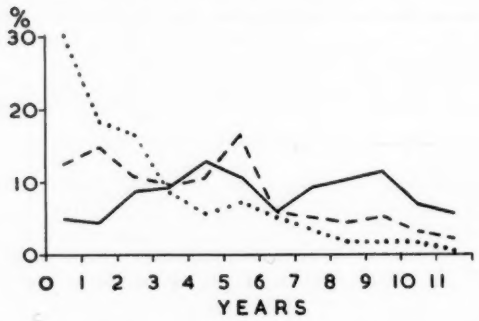

FIGURE I

Age distribution of admissions for asthma (continuous line), for asthmatic bronchitis (broken line) and for bronchitis (dotted line), Brisbane Children's Hospital, 1955–1958. Groups are as defined in Table I. To facilitate comparison, the yearly values for each group are shown as percentages of the total. The second peak in asthmatic bronchitis at five years is probably fortuitous

BRISBANE HOSPITAL CASES

Those patients who resided outside the City of Brisbane or were admitted to hospital for reasons other than an attack of asthma were excluded. As with the children, there were frequent readmissions.

The sex and age distribution is noted in Table III and Figure II. Only 34% of patients (35% of admissions) were male. As males and females were admitted in approximately equal numbers to the Brisbane Hospital, this suggests a female preponderance among adult asthmatics in Brisbane. Authors have reported differently on the sex distribution of asthma (Vaughan and Black, 1954). Bray (1934), referring to age at the first attack, writes as follows:

> Asthma is twice as common in boys as in girls, slightly more prevalent in females than males from the age of puberty to the menopause, after which it is slightly more frequent in males. . . . Puberty must be a definite, decisive milestone towards which a great number of males must improve and beyond which a greater number of females develop symptoms.

The present observations, although on a different basis, agree with those of Bray in regard to the reversal of the sex ratio at puberty and again in later life.

TABLE III

Sex and Age Distribution of Admissions for Asthma from Brisbane City, Brisbane Hospital, 1955–1958[1]

Table IIIA

Statistical Year	Males	Male Percentage	Females	Total
1955–1956	116 (81)	38 (37)	191 (139)	307 (220)
1956–1957	61 (44)	30 (29)	142 (107)	203 (151)
1957–1958	68 (46)	34 (34)	131 (91)	199 (137)
Total	245 (171)	35 (34)	464 (337)	709 (508)

Table IIIB

Age Group (Years)	Males	Male Percentage	Females	Total
12–19 ..	68 (34)	46 (38)	80 (55)	148 (89)
20–29 ..	33 (26)	26 (30)	94 (62)	127 (88)
30–39 ..	35 (24)	28 (26)	89 (68)	124 (92)
40–49 ..	21 (18)	23 (24)	70 (57)	91 (75)
50–59 ..	27 (21)	38 (37)	45 (36)	72 (57)
60–69 ..	34 (26)	34 (37)	65 (44)	99 (70)
70 and over	27 (22)	56 (59)	21 (15)	48 (37)

[1] Figures within parentheses refer to patients.

The oldest patient was a male, aged 86 years, who had had asthma for 15 years, now complicated by emphysema. He was discharged, relieved, after nine days in hospital.

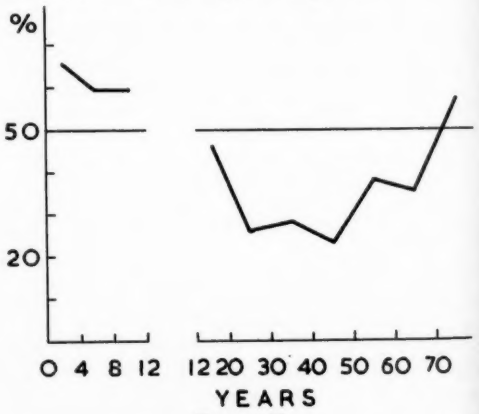

FIGURE II

Male percentage of admissions for asthma to the Brisbane Children's Hospital and Brisbane Hospital at various age groups. There is a striking swing from male to female predominance at puberty, and a return to male predominance in old age

Many occupations were represented; none appeared to have an abnormal frequency.

In 16 cases, the illness ended fatally. The patients were males, aged respectively 22, 38, 62, 65, 67, 73 and 82 years, and females aged respectively 19, 19, 38, 38, 49, 55, 58, 68 and

79 years. Three of these died as they were being admitted to hospital.

The reason for the much higher number of admissions in 1955–1956 than in the other two years is obscure. It is not paralleled in the Brisbane Children's Hospital series.

MONTHLY DISTRIBUTION OF THE BRISBANE CHILDREN'S HOSPITAL ADMISSIONS

The dates used for analysis were the dates on which the attacks began for which the patients were admitted to hospital. When the day of onset was not defined, it was taken to be the day of admission.

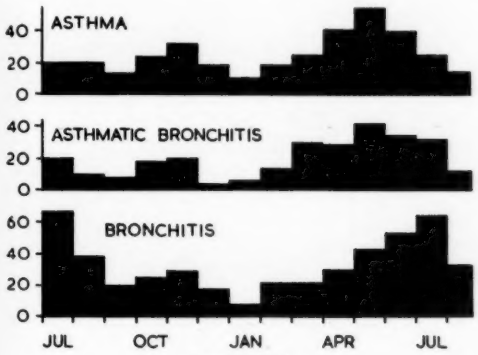

FIGURE III

Monthly admissions to the Brisbane Children's Hospital, 1955–1958, for asthma, asthmatic bronchitis and bronchitis, as defined in Table I. This and subsequent histograms have been continued through the second winter to allow the completion of the autumn-winter wave

The monthly distribution for the asthma, asthmatic bronchitis and bronchitis groups is shown in Figure III. The curves for asthma and asthmatic bronchitis are practically identical. They show a minor wave in the spring months of October and November and a major wave in autumn and early winter, with the peak in May. The similarity of the seasonal distribution of asthma and asthmatic bronchitis was confirmed by the χ^2 test calculated on the monthly totals, the low December and January figures being amalgamated ($\chi^2=11\cdot5$, $df=10$, $P>0\cdot3$).

The common possession of this distinctive seasonal distribution supports the close relation between asthmatic bronchitis and asthma suggested earlier by the frequent alternation of these diagnoses in children repeatedly admitted to hospital. A good index of asthmatic admissions will, therefore, be gained by combining these two groups. Another implication is that the bronchial catarrh that accompanies many attacks of asthma may not be infective, but part of the reaction to the allergen.

The seasonal behaviour of bronchitis was different. The curve had a single peak in mid-winter, and this is suggested as characteristic of bronchitis of infective origin. (In 1893, David Hardie published a similar curve for the bronchitis mortality for all ages for Queensland. The most dangerous months were June, July, August and September, especially July.) The hint of a spring rise is probably due to an allergic origin for some of the bronchitis cases. A comparison of the monthly totals between bronchitis and asthma and between bronchitis and asthmatic bronchitis showed that the differences were highly significant (in each case $\chi^2=34$; $df=10$; $P<0\cdot001$). Bronchial infection cannot explain the increase of asthma in spring and autumn.

The characteristic two-peaked curve of asthma, with some variation, was seen in each of the three years of the study (Figure IV; in this and subsequent analyses, asthmatic bronchitis is included with asthma). The

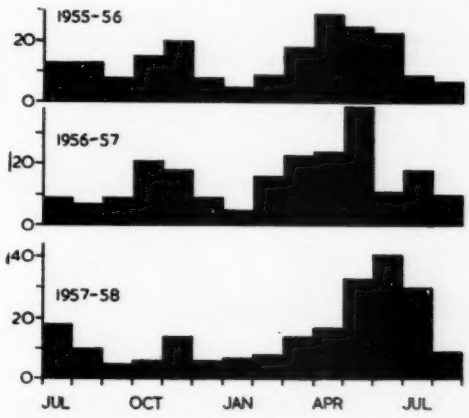

FIGURE IV

Monthly admissions for asthma (including asthmatic bronchitis) to the Brisbane Children's Hospital for each year of the study. The spring and autumn waves occurred in each year, with some variation in height and time

spring wave of 1957 was quite small and, in the monthly totals, evident only in November. The autumn wave of 1958 was late, reaching its peak in June and extending into July. Approximate dates for the beginning and ending of the waves, obtained from the daily record, are shown in Table IV. There was also a slight increase in the daily admissions from July 19 to August 9, 1955, from July 10 to 20, 1957, and from July 22 to 31, 1958, but not in mid-winter,

1956. This mid-winter increase was probably infective in origin.

The distribution was seen to be similar in each sex, and in each age group, except the youngest, when these were analysed separately (Figure V). The curve for the first year of life was different, resembling rather the distribution of infective bronchitis, and reflecting the

TABLE IV

Approximate Duration of Asthma Waves

Year	Autumn-Winter	Spring
1955		October 2 to November 29 (intermittent)
1956	March 23 to June 30	October 12 to November 14
1957	February 20 to June 6	October 27 to November 20 (incidence low)
1958	May 8 to July 12 (intermittent activity from March 1)	

difficulty in the separation of bronchitis from asthma in early infancy. It is likely that some infective cases were among the 16 diagnosed as "asthma" and the 29 as "asthmatic bronchitis" in the first year. Infection probably explains also the high July total in the one to three years group.

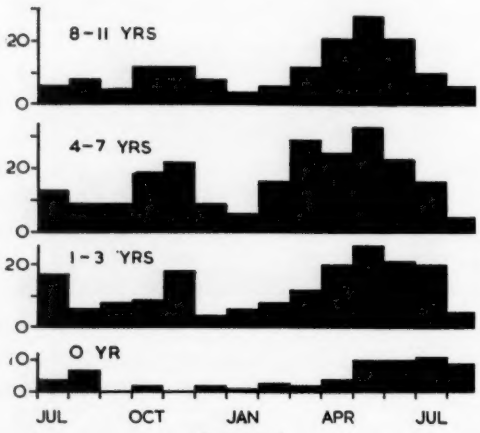

FIGURE V

Monthly admissions for asthma (including asthmatic bronchitis) to the Brisbane Children's Hospital, 1955–1958, analysed by age. The "asthmatic" seasonal pattern prevails at every age after the first year

The monthly distribution was also similar for children from different geographical sections of the city. Thus the spring and autumn waves apply generally throughout the Brisbane area.

An estimate may be made of the relative importance of seasonal factors. If the number of admissions in the three lowest months—September, December and January—is taken to represent approximately the monthly expectancy of apparently non-seasonal cases,

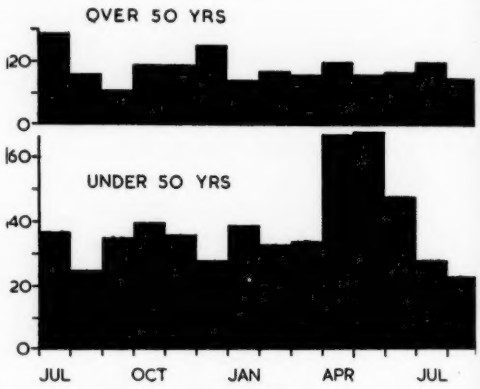

FIGURE VI

Monthly admissions for asthma to the Brisbane Hospital, 1955–1958, analysed by age. Seasonal variation is not evident in the elderly. In those aged under 50 years, an autumn wave is distinct, a spring wave questionable; about 80% of cases are not apparently seasonal

these would amount in the year to about 45% of all admissions. The spring wave would contribute about 10% and the autumn wave about 45%.

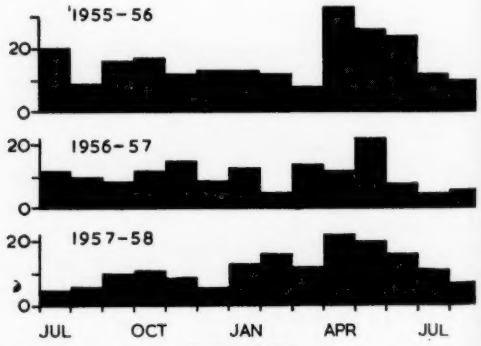

FIGURE VII

Monthly admissions of asthmatics aged under 50 years to the Brisbane Hospital for each year of the study. The autumn wave is consistently present

MONTHLY DISTRIBUTION OF BRISBANE HOSPITAL ADMISSIONS

The Brisbane Hospital cases were divided into two age groups for analysis. For asthmatics aged 50 years and over, there was no consistent seasonal variation (Figure VI). Neither spring nor autumn waves were evident. The high

July and December levels were due to an excess of admissions in those months in one year only.

Admissions of asthmatics aged under 50 years showed a definite autumn wave (Figure VI), but only a faint hint of a spring wave. There was considerable variation from year to year in the shape of the monthly curve (Figure VII), but the autumn wave was consistently present, the peak being in April or May. It was present with both males and females when these were analysed separately. Only about 20% of admissions of asthmatics in this age range belonged to the autumn wave; about 80% were apparently unrelated to season.

The decrease in seasonal variation with age was progressive. The proportion of admissions in the autumn wave in the various age groups was as follows:

Brisbane Children's Hospital:
0 to 11 years: 45%

Brisbane Hospital:
12 to 19 years: 24%.
20 to 29 years: 27%.
30 to 39 years: 19%.
40 to 49 years: 12%.
50 years and over: 0.

This implies that the ætiological factors responsible for the autumn wave in childhood become less operative with increasing age.

ASTHMA AND THE WEATHER

A relation of attacks to the weather is a recurrent theme in reports of asthma. Feinberg (1944) concludes:

> Weather conditions play a very important role in the causation of asthma. . . . A marked fall in temperature, a sudden barometric change, and stormy rainy weather are the changes which evoke an epidemic of asthma.

However, opinions on the type of weather responsible are diverse, and the mechanism of the suspected relation is often obscure. In this series, the mother of Gregory, aged three years, ascribed his attacks to cool changes in the weather. "Changeable weather" also brought on asthma with Desmond, aged nine years. Roslyn, aged two years, had attacks when it rained. The commencement of westerly winds was blamed by one practitioner.

In an attempt to define a relation to the weather, correlation coefficients were calculated between the number of monthly admissions of children with asthma (including asthmatic bronchitis) and monthly values for the elements of the weather (Tables V, VI). The children were more suitable than the adults for this comparison because of the greater seasonal variation. The coefficients were calculated for each twelve-month period separately, and also for the 36 months as a whole.

The best correlation in Table V is that (negative) with wind speed. This tends to be high in summer and low in winter, but with much variation from year to year in the monthly pattern (Figure VIII). The correlation was significant in 1955–1956 and in the combined series, and almost so in 1957–1958. However, this association cannot be fundamental, for it failed completely in 1956–1957. The suggestive correlations with 9 a.m. relative humidity and sunshine in 1957–1958 are discounted because of lack of support in the other years.

TABLE V

Correlation of Monthly Admissions to Brisbane Children's Hospital for Asthma (Including Asthmatic Bronchitis) with Elements of Weather

Elements of Weather	1955–1956	1956–1957	1957–1958	1955–1958
Barometric pressure, 9 a.m.	0·10	0·15	0·41	0·19
Dry-bulb temperature, daily maximum	−0·27	0·18	−0·52	−0·23
Dry-bulb temperature, daily minimum	−0·22	0·04	−0·32	−0·17
Dry-bulb temperature, daily mean	−0·25	0·11	−0·42	−0·20
Dry-bulb temperature, daily range	−0·16	0·30	−0·42	−0·11
Dry-bulb temperature, 9 a.m.	−0·24	0·07	−0·50	−0·24
Wet-bulb temperature, 9 a.m.	−0·27	0·03	−0·33	−0·19
Dew point, 9 a.m.	−0·26	0·05	−0·15	−0·11
Relative humidity, 9 a.m.	−0·10	−0·11	0·71[1]	0·27
Relative humidity, 3 p.m.	−0·23	−0·26	0·40	−0·01
Relative humidity, 9 p.m.	−0·25	−0·12	0·44	0·02
Sunshine, hours per day	0·00	0·16	−0·66[1]	−0·25
Wind speed, miles per day	−0·65[1]	−0·05	−0·53	−0·40[1]
Rainfall, monthly total[2]	−0·22	−0·26	0·50	0·00

[1] $P<0·05$. (At the 0·05 level of probability, a coefficient of 0·576 would be significant for the twelve-month correlation, and one of 0·33 for the thirty-six-month correlation.)

[2] For rainfall, the monthly totals were used for correlation. For the other elements, the monthly mean was used.

TABLE VI

Correlation of Monthly Admissions to Brisbane Children's Hospital for Asthma (Including Asthmatic Bronchitis) with Wind Direction

Direction of Wind	1955–1956	1956–1957	1957–1958	1955–1958
Northerly	0·10	−0·16	−0·40	−0·21
Easterly	−0·57	0·04	−0·71[1]	−0·44[1]
Southerly	−0·40	−0·08	0·17	−0·08
Westerly	0·54	0·06	0·44	0·35[1]

[1] $P<0·05$. P values as in Table V.

The direction of the wind is recorded three times daily at the Brisbane Weather Bureau. Sixteen directions are distinguished. These have been reduced for analysis to four. Thus north-west, north-north-west, north and north-north-east have been grouped as northerly, and so on round the compass. The index of each

wind direction used for correlation was the number of observations monthly of that direction.

Asthma was often associated with westerly winds (Table VI). There was significant correlation in the combined series, and correlation approaching significance in 1955–1956 and 1957–1958. However, there was no correlation in 1956–1957. Correspondingly, significant negative correlations were found with easterly winds. It is suggestive that the predominance

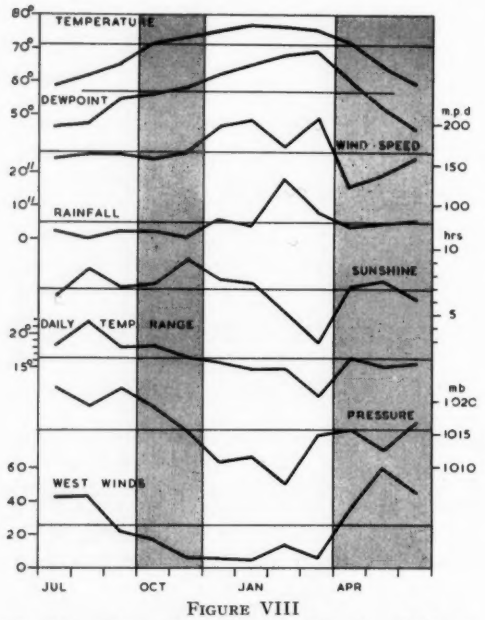

FIGURE VIII

Monthly values for weather elements, Brisbane, 1955–1956. Means are indicated by horizontal lines. The essentially single-peaked distribution may be contrasted with the double-peaked distribution of asthma admissions in childhood

of westerly winds usually begins, like the major asthma wave, in autumn and continues through the high asthma months of May and June (Figure VIII). But some other factor is more important in the production of asthma, for westerlies also predominate in the low asthma months of July and August and are rare during the October-November asthma wave. That other factor is probably the amount of allergens carried. In Brisbane, this is likely to be much greater with westerlies that have blown over the land than with easterlies from the sea. That westerly winds are not of primary importance is shown by the absence of correlation in 1956–1957. In that year, the autumn asthma wave began unusually early (February) and prevailed for several months before the westerlies began to predominate in May.

It is undeniable that a function that varies with the seasons must have some association, direct or indirect, incidental or causative, with the weather complexes that accompany seasonal changes. Some points in the association of asthma and the weather are clear. Thus pollen production requires certain conditions of sunshine and rain at the relevant times; a change of wind may flood a locality with pollens or drive them away; temperature inversion may concentrate air-borne allergens (Morrison and Bath, 1960); a cold change may precipitate attacks in a child sensitive to cold; warmth and moisture encourage the multiplication of fungi.

Any effects of sudden changes in the weather would not be disclosed in correlations based on monthly values, nor were our calculations designed to bring out associations with a complex of weather elements; but it may safely be concluded from Tables V and VI that the characteristic seasonal distribution of asthma in Brisbane is not to be explained by reference to any single element of the weather.

This conclusion is supported by the contrast in distribution curves. The distribution of the main weather elements is essentially single-peaked, except for irregularities which vary from year to year (Figure VIII). It is difficult to conceive of a close relation between this type of curve and the double-peaked curve of asthma.

It seems likely that the influence of weather changes on the incidence of asthmatic attacks is not primary, but depends mainly on whether they concentrate or disperse air-borne allergens.

Interpretation of the Seasonal Distribution

A concept of the nature of asthma in childhood is that most cases are basically allergic; at times attacks may be precipitated by non-allergic factors such as cold, infection, emotion, or pulmonary irritants, as well as by simple exposure to allergens. The seasonal incidence of asthma in Brisbane may be reviewed in relation to these ætiological factors. It has been noted that the spring and autumn waves do not correspond with the coldest weather, or with a high incidence of bronchitic infection. There is no tendency for emotional disturbances or pulmonary irritants (paint fumes, smoke, soap powder, etc.) to intensify in spring and autumn. We are left with allergens. The allergens most strikingly related to season are pollens, and it is probable that the spring and autumn waves are mainly due to a prevalence at those times of allergenic, air-borne pollens. Asthma due to air-borne fungi is also seasonal; the peak would be expected in late summer,

when temperature and humidity are greatest. Further studies will be necessary to determine the relative importance of the various allergens. No reports on pollens or fungi in relation to allergy in Brisbane have yet been published.

ACKNOWLEDGEMENTS

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PRIMARY ALDOSTERONISM—CONN'S SYNDROME

SOME OBSERVATIONS ON THE EFFECT OF AN ALDOSTERONE ANTAGONIST, SPIRONOLACTONE, AND VASOPRESSIN[1]

F. O. SIMPSON[2] and A. J. BARNETT[3]

From the Baker Medical Research Institute, Alfred Hospital, Melbourne

SUMMARY

Electrolyte-balance studies on a patient with primary aldosteronism are reported.

An aldosterone antagonist, spironolactone ("SC 9420", "Aldactone", Searle), was effective in promoting sodium excretion and, to some extent, potassium retention.

Spironolactone had some effect also after removal of the tumour.

Vasopressin caused an increase in urine flow in this case.

THE importance of aldosterone is well recognized, both in the normal regulation of water and electrolyte balance and also in certain pathological conditions, such as Conn's syndrome (primary aldosteronism) and various œdema states (in some of which there is "secondary" aldosteronism). However, the mode of control of the hormone and its precise mode of action are still not fully understood.

An opportunity to investigate certain aspects of the problem has been provided by the recent development of a series of aldosterone antagonists, the 17-spirolactones (Cella and Kagawa, 1957). The effect of one of these, "SC 8109" (Searle) on patients with primary aldosteronism has been reported (Salassa *et alii*, 1958; Bolte *et alii*, 1958; Conn *et alii*, 1958), and in the present paper we describe the use of a similar drug, spironolactone ("SC 9420," "Aldactone," Searle) in another such case. We also report an anomalous diuretic effect of vaso-pressin in the same case.

CASE HISTORY

The patient was a woman with primary aldosteronism due to a benign adrenocortical adenoma. She also presented some of the features of Marfan's syndrome. Her clinical history is reported more fully elsewhere (Simpson and Barnett, 1960), and is briefly summarized below.

A female patient, aged 37 years, was found to have a raised blood pressure after her first pregnancy at the age of 26 years. Headaches and more severe hypertension developed after her second pregnancy at the age of 30 years, and lumbo-dorsal sympathectomy was performed. Her third pregnancy (at the age of 35 years) was terminated at 37 weeks on account of hypertension. Six weeks after delivery she experienced muscle weakness lasting for several weeks. Other symptoms were moderate polyuria and polydipsia, minor episodes of left ventricular failure and slight ankle œdema in hot weather. Her blood pressure was 240/130–140 mm. Hg. She was treated with chlorothiazide and reserpine, and her blood pressure fell; but severe muscle weakness and hypokalæmia developed, and Conn's syndrome was suspected. The urinary aldosterone excretion was found to be 50 μg. in 24 hours,[1] and a left suprarenal tumour was demonstrated radiologically. Excision of this tumour, which proved to be a benign adrenocortical adenoma, was followed by a temporary fall in blood pressure and a long-lasting correction of her biochemical abnormalities.

OBSERVATIONS ON ELECTROLYTE BALANCE, INCLUDING THE EFFECT OF THE ALDOSTERONE ANTAGONIST, SPIRONOLACTONE

Methods

There was a delay of several months, for domestic reasons, between the time of diagnosis and the operation. During this period the patient was treated as an out-patient with mecamylamine, reserpine and potassium chloride (40 mEq per day).

She was eventually admitted to hospital four weeks before operation, and during her stay in hospital an electrolyte balance study was performed, the intake and output of sodium, potassium and chloride being determined daily. The intake figures were obtained by assessing the individual items of diet and are therefore only approximate. During most of the time, a normal diet was given, containing 60 to 90 mEq of sodium, 55 to 75 mEq of potassium, and 50 to 75 mEq

[1] Received on April 7, 1960.

[2] Edward Wilson Memorial Research Fellow. Present address: Department of Medicine, University of Otago, Dunedin, New Zealand.

[3] Associate Director.

[1] Estimation by Dr. J. Bornstein, Diabetic and Metabolic Unit, Alfred Hospital.

of chloride per day; however, a diet of low sodium content (28 mEq per day) was given for two days, and there was also a period of low electrolyte intake while the patient was anorexic immediately after operation. The potassium chloride supplement of 40 mEq per day was continued at first; it was increased to 80 mEq per day for five days, and then

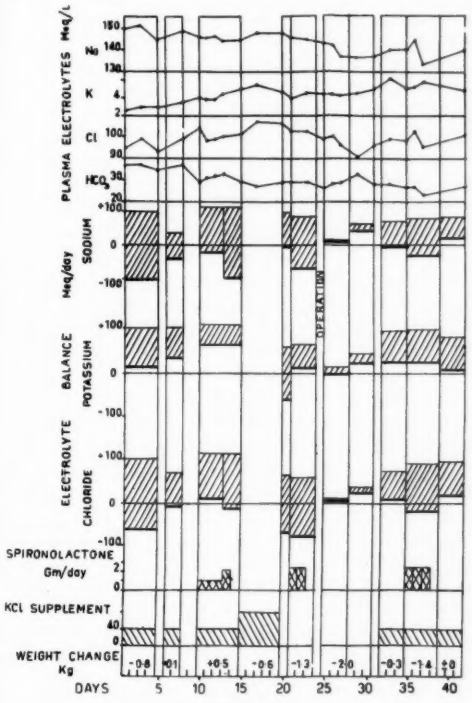

FIGURE I

Chart of electrolyte balances and plasma electrolyte levels. Electrolyte intake is plotted above the zero balance lines. Output is plotted downwards from the line representing intake. The extent of the positive or negative balance is shown by the position of the heavy line above or below the zero line

discontinued in the immediate pre-operative and post-operative periods. The average daily electrolyte intake during the various study periods is shown in Figure I.

Plasma electrolyte levels were estimated frequently during the patient's stay in hospital.

Spironolactone was administered for three short periods (see Figure I), as only small supplies were available. Pre-operatively, it was given on the first occasion in a dosage of 1 gramme per day for three days and 2·4 grammes on the fourth day, and on the second occasion in a dosage of 2·5 grammes per day for two days. Post-operatively, it was given on one occasion only, in a dosage of 2·5 grammes per day for three days.

The patient was also weighed daily, and her intake of fluid and output of urine were measured.

Results

The electrolyte balance and plasma electrolyte levels are shown graphically in Figure I. The balance study was unsatisfactory for technical reasons on certain days, which are shown as gaps on the chart. The whole period of five days during which 80 mEq of potassium chloride were given daily has been omitted, as the patient suffered from severe anorexia, nausea and vomiting during this time. Unfortunately, it was not possible to prolong the study to allow repeated control periods. However, certain facts emerge from the study.

Days 1 to 4: When the patient was admitted to hospital, the plasma potassium content was only 2·5 mEq/l., despite the previous daily potassium chloride supplement of 40 mEq. However, from the time of her admission, she showed a negative sodium balance and a positive potassium balance.

Days 6 to 10: A short period of restriction of sodium intake did not affect potassium balance; sodium loss was less than in the initial period. In the 10 days from the time of her admission until the first course of spironolactone was started, the plasma potassium level rose from 2·5 to 4·0 MEq/l.

Days 10 to 14: The low initial dosage of spironolactone (1·0 gramme per day) had no demonstrable effect on sodium excretion, and in fact on the third day of this dosage there was actually a slightly positive sodium balance. However, when the dose was increased to 2·4 grammes on the fourth day, there was a very large loss of sodium, which continued on the following day. Potassium retention was more marked during spironolactone administration, and the plasma potassium level rose to 5·1 mEq.

Days 15 to 20: The increase in the potassium chloride supplement to 80 mEq per day caused nausea and vomiting to such an extent that balance studies were impossible; however, on the day after the supplement had been stopped there was a considerable net loss of potassium.

Days 21 to 23: The second course of spironolactone administration (2·5 grammes per day for two days) was associated with a considerable sodium loss, and the potassium balance again became positive, although no potassium supplement was being given at this time.

Day 24: A benign adreno-cortical adenoma was removed.

Days 25 to 30: In the post-operative period there was at first electrolyte equilibrium, and subsequently there were positive sodium and potassium balances.

Days 32 to 34: The addition of a daily potassium chloride supplement of 40 mEq did not increase the net potassium gain, but sodium excretion increased, so that there was sodium equilibrium during this period.

Days 35 to 38: Spironolactone in a dosage of 2·5 grammes per day for three days also did not alter the potassium balance, but it was associated with a further increase in sodium excretion resulting in a negative sodium balance on these three days and on the following

day. The average daily loss of sodium was approximately half of that produced by a similar dose of spironolactone before operation.

Days 39 to 41 : After the withdrawal of spironolactone, the sodium balance again became positive, and the potassium balance fell towards equilibrium.

In the early stages of the study the patient's weight was somewhat variable. It was during this time that water-excretion studies were being performed with water loads and vasopressin. However, there were subsequently three periods of definite weight loss : in the post-operative period, and during the second and third courses of spironolactone, when negative water balances were also recorded. The spironolactone therefore appeared to have a diuretic effect both before and after removal of the aldosterone-producing tumour.

The results of the electrolyte balance studies can be summarized as follows :

(1) Partial rest in bed in hospital was associated initially with a markedly negative sodium balance.

(2) One gramme per day of spironolactone had no effect on the electrolyte balance, whereas 2·5 grammes per day had a definite effect.

(3) During spironolactone administration, there was retention of potassium before operation, but not after operation.

(4) During spironolactone administration, there was a loss of sodium both before operation and to a lesser degree after operation, and there was a possibility of a " rebound " retention of sodium on withdrawal of the drug.

(5) During spironolactone administration, there was a diuresis, a negative water balance and a loss of weight both pre-operatively (on one occasion) and post-operatively.

Discussion

It is remarkable that the patient's plasma potassium level rose so rapidly from 2·5 to 4·0 mEq/l. in the first 10 days after her admission to hospital, and that there was such a large loss of sodium during this period. The potassium supplement (40 mEq per day) had been given on an out-patient basis, and had not been altered on her admission to hospital. The possibility that she had failed to take the supplement as an out-patient is unlikely, as she was a most cooperative and reliable patient. Other possibilities are that the potassium taken in the early stages was largely used in replenishing cells without affecting the plasma level, or that the altered circumstances associated with admission to hospital facilitated the retention of potassium. The hospital diet probably did not differ greatly from her diet at home, and the injections of vasopressin were not associated with particularly large daily losses of sodium. The main change in her circumstances on her admission to hospital was that she was rested and was recumbent for a large part of the day. It has been shown (Muller *et alii*, 1958) that aldosterone output in normal subjects is higher by day than by night, provided that the subject is up during the day, and also that sodium excretion increases when the subject is kept in bed ; but no reports have been found of similar studies on patients with primary aldosteronism. It is possible that a similar phenomenon took place in the present case ; this would imply some degree of a normal control mechanism over aldosterone production by the tumour, but the evidence is not conclusive.

There was no doubt of the effectiveness of spironolactone in promoting the excretion of sodium in the present case when it was given in adequate dosage (2·5 grammes per day). The series of 17-spirolactones to which spironolactone belongs are thought to block the action of aldosterone and desoxycorticosterone on the renal tubules by competing with the natural hormones (Kagawa *et alii*, 1957), thus causing a sodium diuresis. They are not effective in promoting sodium excretion in normal rats and dogs, (Kagawa *et alii*, 1959), in patients with Addison's disease, or in normal human subjects who are taking a diet of high sodium content (Liddle, 1957) or a normal diet (Ross and Bethune, 1959) ; but they do cause an increase in sodium output in normal human subjects on a diet of low sodium content when aldosterone production is increased (Liddle, 1957) and in various pathological states.

Previous reports on the use of spirolactones in cases of primary aldosteronism have shown somewhat dissimilar results. Salassa *et alii* (1958) assessed the effect of the spirolactone " SC 8109 " (Searle) in a case of primary aldosteronism. They found that 1 gramme per day had only a slight effect, but 1·9 grammes per day a marked effect. The sodium balance, which in the base-line period was slightly positive, became strongly negative, and the potassium balance, which was also slightly positive, became more strongly positive. They found that the drug had no effect when it was administered after removal of the aldosterone-producing tumour. The same authors found an increase in aldosterone output during periods of spirolactone administration ; it is possible that this is a compensatory phenomenon, which may account for the retention of sodium

seen in the present case after withdrawal of spironolactone on one occasion. Bolte *et alii* (1958) also used "SC 8109" in a case of primary aldosteronism, giving 1·2 grammes per day for two days. They found an increase in sodium excretion, but no increase in aldosterone output. Kistler *et alii* (1959) used "SC 8109" (4·8 grammes in four days) in the treatment of a hypertensive patient in whom mineralocorticoid excess was suspected, but whose aldosterone excretion was normal; the urinary sodium: potassium ratio increased, and the serum potassium level rose. At operation, this patient was found to have nodular hyperplasia of the zona glomerulosa, and she would appear to fall into the category of primary mineralocorticoid excess.

Conn *et alii* (1958), using "SC 8109" in a case of primary aldosteronism, found evidence suggesting aldosterone antagonism only when the patient was on a diet of very low sodium content. They did not find a similar effect when the patient was on a diet of high sodium content, although the output of aldosterone was unaltered, and they conclude that "SC 8109" reverses the effects of endogenous aldosterone only in certain circumstances.

The compound used in the present case, spironolactone, has been found by Kagawa (1959) to be five times as active as "SC 8109" when administered orally to rats, but to have only 0·27 of the activity of "SC 8109" when administered subcutaneously. However, as far as can be judged from our results in this case, compared with the results of Salassa *et alii* (1958) and of Bolte *et alii* (1958), spironolactone is not more potent than "SC 8109" when administered orally in man, but the cases are not necessarily comparable. It is difficult in the present case to account for the effectiveness of spironolactone in promoting sodium excretion after removal of the adrenocortical adenoma, at a time when the patient was having a normal sodium intake. A potassium supplement was being given, but aldosterone output should still have been very low. The presence of a second aldosterone-producing tumour is a remote possibility, as cases of Conn's syndrome with multiple adrenocortical adenomata have been described (Thorn *et alii*, 1957; Mucio *et alii*, 1958).

Observations on the Effect of Vasopressin on Urine Volume and Specific Gravity

Methods

The observations were made during the early part of the patient's stay in hospital, when she was taking a normal diet (apart from two days of a sodium-poor diet) with a potassium supplement of 40 mEq per day, and was in positive potassium balance and negative sodium balance. Her plasma potassium level rose from 2·9 to 3·7 mEq/l. during the week when these observations were made. She tended to have moderate polyuria, passing usually 1800 to 2500 ml of urine daily. The specific gravity varied, values up to 1·020 being found, although lower values were usually obtained during formal water deprivation tests.

Test A: Effect of Vasopressin on Excretion of a Water Load.—No fluid was given between 7 p.m. and 7 a.m. A water load of 1450 ml. was given orally at 7 a.m. A subcutaneous injection of either vasopressin ("Pitressin", Parke Davis, 10 units), or 2 ml. of 0·9% saline as a control, was given at 7 a.m. Subsequently the urine was collected and the volume and specific gravity were measured. (See Table I.)

Test B: Effect of Vasopressin on Urine Flow in Absence of a Water Load.—A similar procedure to Test A was adopted, except that one cup of tea was given in place of the water load. (See Table I.)

Test C: Effect of Vasopressin on Excretion of a Water Load after a Renal Clearance Study.—After completion of a renal clearance study (sucrose and *P*-aminohippurate clearances), during which frequent urine specimens were obtained with an indwelling catheter, a subcutaneous injection of vasopressin ("Pitressin", 10 units) was given. Specimens of urine were taken at intervals thereafter, and measurements were made of the volumes and specific gravity.

Attempts were made to repeat these observations two months after adrenalectomy, but the patient appeared to have become sensitized to "Pitressin." She experienced vomiting and diarrhœa on each of three occasions when 10 units were injected, so that it was impossible to obtain satisfactory observations.

TABLE I

Effect of Vasopressin on Urine Flow

Date	Water Load (Oral)	Injection	Urine Output	Specific Gravity
April 7, 1959	1450 ml.	2 ml. of 0·9% saline	308 ml. in 4 hours	1·004–1·010
April 8, 1959	1450 ml.	Vasopressin, 10 units	1340 ml. in 3 hours	1·004–1·006
April 12, 1959	1450 ml.	2 ml. of 0·9% saline	409 ml. in 4 hours	1·005–1·013
June 14, 1959	150 ml.	Vasopressin, 10 units	210 ml. in 2 hours	1·005
June 15, 1959	150 ml.	2 ml. of 0·9% saline	40 ml. in 2 hours	1·015

Results

In Test A (see Table I), on the days when control injections were given, urine volumes were small—only 308 ml. and 409 ml. respectively were passed in four hours. However, on the day when vasopressin was given, the urine volume was 1340 ml. in three hours. Its specific gravity was higher on the control injection days (1·010 and 1·013 respectively) than on the day of the vasopressin injection (1·006).

In Test B (see Table I), the two-hour urine volume was 40 ml (specific gravity 1·015) on the day of the control injection, and 310 ml. (specific gravity 1·005) on the day when vasopressin was given.

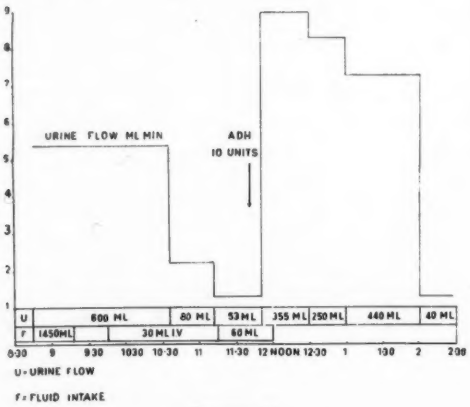

FIGURE II

Chart of urine flow in response to a subcutaneous injection of vasopressin, 10 units

In Test C (see Figure II), the initial water load had not been fully excreted by the time vasopressin was given, and yet urine flow had fallen to a level of 1·3 ml per minute. After a small second water load and the injection of vasopressin, the urine flow increased to 9 ml. per minute and remained at a high level for about two hours, after which it fell to a low level again.

In all these tests, therefore, urine flow was considerably greater after administration of vasopressin than in the comparable control period.

DISCUSSION

Failure of urinary specific gravity to rise in cases of primary aldosteronism after administration of vasopressin was first noted by Conn in his original case (Conn, 1955), and this has been confirmed in other cases of the syndrome (Alsted and Halberg, 1958; Barrett *et alii*, 1958; Bartter and Biglieri, 1958; Brooks *et alii*, 1957; Van Buchem *et alii*., 1956; Chalmers *et alii*, 1956; Eales and Linder, 1956; Foye and Feichtmeir, 1955; Hudson *et alii*, 1957; Siguier *et alii*, 1958; Skanse *et alii*, 1957; Therian, 1958). The effect of vasopressin on urinary volume is usually not mentioned in these case reports; but in the previous case from this unit (Hudson *et alii*, 1957) and in the case of Skanse *et alii* (1957) there was a reduction in the urine volume without a rise in urinary specific gravity. It is generally considered that this "'Pitressin' resistance" is due to the effect of potassium deficiency on the kidney. Similar effects have been noted in patients with potassium deficiency due to chronic diarrhœa, as occurs after prolonged misuse of purgatives (Relman and Schwartz, 1955), and in animals rendered potassium-deficient by prolonged DCA administration (Ragan *et alii*, 1940), or by potassium-free diets (Hollander *et alii*, 1957; Richter 1958).

The anomalous diuretic effect of vasopressin, which was found in the present case, does not appear to have been noted previously in man. However, Richter (1958) found, in potassium-depleted rats, that three different types of response to vasopressin occurred, depending on the degree of potassium depletion. In rats with slight potassium depletion there was a small normal response with a slight increase in specific gravity and decrease in urine flow; in rats with moderate potassium depletion there was no response; and in rats with the most severe depletion there was an actual increase in urine flow after vasopressin administration. Our patient therefore corresponds to the rats with the most severe potassium depletion in her response to vasopressin. It is curious that this type of response has not occurred in other patients, some of whom have had lower plasma potassium levels than our patient, and the mechanism of the phenomenon is not clear. The only similar effect noted in the cases reviewed recently (Simpson and Barnett, 1960) was in Van Buchem's (1956) patient, who appears from the published graph to have had a small increase in urine volume after vasopressin.

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THYROID-STIMULATING HORMONE AND TRIIODOTHYRONINE AS AIDS IN THE DIAGNOSIS OF THYROID DISORDERS[1]

BRYAN HUDSON,[2] DORA WINIKOFF,[3] H. PINCUS TAFT[4] and F. I. R. MARTIN[5]
From the Diabetic and Metabolic Unit, Alfred Hospital, Melbourne, and the Royal Melbourne Hospital

SUMMARY

The administration of thyroid-stimulating hormone (T.S.H.) and 3 : 5 : 3′-triiodothyronine (T_3) has been applied in the assessment of thyroid function in cases of doubtful hypothyroidism, in myxœdema of uncertain origin and in cases of suspected hyperthyroidism. The indices used were plasma protein-bound iodine (P.B.I.) and I^{131} uptake by the thyroid.

After T.S.H., the rise in P.B.I. levels was greatest in euthyroid patients (regardless of previous thyroid administration), least in primary thyroid failure and intermediate in pituitary myxœdema. The rise in I^{131} uptake after T.S.H., on the other hand, distinguished only the euthyroid group from patients with primary myxœdema, being significantly higher in the former group.

After T_3 administration, suppression of I^{131} uptake, regardless of the initial value, was observed in the euthyroid group, whilst in thyrotoxicosis no such suppression occurred. Changes in P.B.I. levels were of no diagnostic significance in these cases.

DURING the past 15 years there has been steady development in methods of investigation of thyroid function. Later techniques include the measurement of plasma hormonal iodine (protein-bound iodine—P.B.I.) and various indices based on measurements of radioactivity following administration of radioactive iodine (I^{131}).

In the majority of cases, accurate diagnosis may be made by the application of one or more of these tests in conjunction with the clinical assessment of the patient. There are, however, circumstances in which these methods are still inadequate. These may be set out as follows: (i) when thyroid medication has invalidated the results of I^{131} studies and P.B.I. estimations, by suppressing the former and elevating the latter; (ii) when uncertainty exists as to whether hypothyroidism has its origin in primary thyroid insufficiency or has arisen as the result of pituitary failure; (iii) when clinical diagnosis is uncertain, and contradictory results are given by the more commonly used tests of thyroid function.

[1] Received on December 10, 1959.
[2] Assistant Physician, Diabetic and Metabolic Unit, Alfred Hospital, Melbourne.
[3] Research Fellow, Diabetic and Metabolic Unit, Alfred Hospital, Melbourne.
[4] Physician in Charge, Diabetic and Endocrine Clinic, Royal Melbourne Hospital, Melbourne.
[5] George Merriman Research Fellow, Diabetic and Metabolic Unit, Alfred Hospital, Melbourne.

In such cases, valuable assistance in diagnosis may be obtained by the use of thyroid-stimulating hormone (T.S.H.) or by 3 : 5 : 3′-triiodothyronine (T_3) administration followed by the measurement of I^{131} uptake by the thyroid, by estimation of the plasma P.B.I., or both. In this paper, our combined experience with these procedures over the past six years is described.

MATERIALS AND METHODS

Clinical Material

Fifty-six subjects, including seven normal controls, have been studied. These patients were referred because of suspected thyroid disease, and all but four have been examined by at least one of us. As was indicated in the introductory paragraphs, we studied three groups of clinical problems, as follows: (i) Patients who had previously been regarded as hypothyroid, but in whom a doubt existed as to the diagnosis. Some had been receiving thyroid medication for months or years prior to this investigation. (ii) Patients with unequivocal features of myxœdema, when it was uncertain whether the subthyroid state was the result of thyroid or pituitary failure (primary or secondary hypothyroidism). (iii) Patients with suspected hyperthyroidism and high I^{131} uptake but normal P.B.I. levels.

All patients were instructed to follow a diet calculated to contain less than 20 μg. of iodine per day for seven to 10 days prior to investigation.

Thyroid-Stimulating Hormone

"Ambinon B" (Organon) was used in every instance. Four intramuscular injections, each of 1 ml., were given at twelve-hourly intervals, the first at 48 hours and the last at approximately 12 hours prior to the measurement of I^{131} uptake or P.B.I.

3 : 5 : 3'-Triiodothyronine

"Cynomel" (Smith, Kline and French) was administered orally in doses of 25 μg. three times a day for seven to 10 days prior to investigation. The iodine contained in this dose does not contribute to the levels of endogenous iodine as measured in P.B.I.

I^{131} Uptake

This was measured four hours after the administration of a 50 μc. tracer dose of I^{131}. The methods of measurement and normal values have been previously described (Hudson and Martin, 1959).

Determination of Plasma P.B.I.

The distillation technique of Barker was used. The results obtained from this procedure have been described elsewhere (Winikoff, 1954).

Other Procedures

The diagnosis of hypopituitarism was made by the conventional methods of clinical history, physical examination, X-ray examination, and 17-ketosteroid, 17-hydroxysteroid and F.S.H. excretion studies.

RESULTS

T.S.H.-Induced Changes in Plasma P.B.I. Levels in Euthyroidism and Primary and Secondary Hypothyroidism

Table I represents a group of euthyroid patients, including three normal controls and eight in whom the possibility of thyroid deficiency was originally suspected on clinical grounds. The range of change in plasma P.B.I. level after the administration of T.S.H. was 2·2 μg. to 10·3 μg. per 100 ml., the mean rise being +5·3 μg. per 100 ml.

In Table II is recorded the response of plasma P.B.I. level to T.S.H. administration in 11 cases in which primary myxœdema was clinically diagnosed, and in which this diagnosis was subsequently confirmed. The range of change here was from 0·0 μg. to 1·8 μg. per 100 ml., the mean rise being +0·7 μg. per 100 ml.

Table III shows the alteration in P.B.I. levels after T.S.H. administration to 11 patients with clinical evidence of hypopituitarism, their diagnosis being confirmed on other grounds. Changes in P.B.I. level after T.S.H. administration were 0·6 μg. to 6·0 μg. per 100 ml., the mean rise being +2·6 μg. per 100 ml.

TABLE I

The Response of the Plasma P.B.I. Level of 11 Euthyroid Subjects to T.S.H. Stimulation[1]

Patient	P.B.I. Level (Microgrammes per 100 ml.)[2]			Remarks
	Initial	After T.S.H.	Difference	
M.McL.	4·1	6·3	+ 2·2	Normal control
H.P.T.	4·6	8·4	+ 3·8	Normal control
F.D.	4·4	11·8	+ 7·4	Normal control
J.D. ..	1·8	5·2	+ 3·4	Anorexia nervosa
M.McK.	4·6	10·2	+ 5·6	Anorexia nervosa
G.D.	3·8	10·0	+ 6·2	Primordial dwarf.
J.T. ..	5·1	15·4	+10·3	Euthyroid — ? hypothyroid
R.B.	4·6	12·2	+ 7·6	Euthyroid — ? hypothyroid
J.K. ..	5·3	8·9	+ 3·6	Euthyroid — ? hypothyroid
F.J. ..	4·5	8·3	+ 3·8	Euthyroid — ? hypothyroid
L.D. ..	5·4	7·9[1]	+ 2·5	Euthyroid — ? hypothyroid

[1] In all patients except L.D., the after T.S.H. determination was performed at 48 hours after the first dose of T.S.H.; for L.D. the value is after 72 hours.
[2] Mean difference in P.B.I. level, +5·3 μg per 100 ml.

It can be seen that the response of the thyroid as measured by increase in P.B.I. levels is greatest in the normal subject, least in primary hypothyroidism and intermediate in hypothyroidism of pituitary origin.

TABLE II

The Response to T.S.H. of the Plasma P.B.I. Level in 11 Cases of Hypothyroidism due to Primary Thyroid Disease

Patient	P.B.I. Level (Microgrammes per 100 ml.)[1]			Remarks
	Initial	After T.S.H.	Difference	
M.T. ..	5·8	6·6	+0·8	Addison's disease; thyroidectomy
A.S. ..	0·6	1·0	+0·4	Myxœdema
J.F. ..	1·4	0·7	−0·7	Myxœdema
T.Y. ..	2·9	2·8	−0·1	Surgical hypothyroidism
J.H. ..	5·1	6·7	+1·6	Myxœdema; receiving thyroid
M.S. ..	2·0	2·5	+0·5	Myxœdema
K.S. ..	0·8	1·0	+0·2	Cretinism; receiving thyroid
C.E. ..	1·4	1·4	±0·0	Dwarfism
J.D. ..	2·1	1·9	−0·2	Dwarfism
M.S. ..	0·7	0·7	±0·0	Myxœdema
W.M.	1·6	3·2	+1·6	Myxœdema

[1] Mean difference in P.B.I., +0·7 μg per 100 ml.

T.S.H.-Induced Changes in I^{131} Uptake in Euthyroidism and Primary and Secondary Hypothyroidism

Table IV shows the increase in I^{131} uptake in seven patients suspected of hypothyroidism who were ultimately classified as euthyroid. The difference in I^{131} uptake ranged from 15·7% to 46·3%, the mean rise being +29·3%.

TABLE III

The Response to T.S.H. of the Plasma P.B.I. Level of 11 Patients with Hypopituitarism[1]

Patient	P.B.I. Level (Microgrammes per 100 ml.)[2]			Remarks
	Initial	After T.S.H.	Difference	
D.D. ..	4·5	5·8	+1·3	Sheehan's syndrome
F.J. ..	4·3	6·6	+2·3	Sheehan's syndrome
M.B. ..	0·5	3·7	+3·2	Sheehan's syndrome
L.McM.	2·8	5·2	+2·4	Chromophobe adenoma
J.A. ..	1·5	3·9	+2·4	Sheehan's syndrome
M.D. ..	3·0	5·3	+2·3	Sheehan's syndrome
M.S. ..	3·7	6·6	+2·9	Sheehan's syndrome
J.F. ..	1·1	1·7	+0·6	Sheehan's syndrome
J.O'S.	2·9	8·9	+6·0	Sheehan's syndrome
H.D. ..	3·8	8·0	+4·2	Hypopituitarism; cause unknown
J.P. ..	3·0	3·8	+0·8	Ependymoma (deep X-ray therapy)

[1] In all patients the maximal response was observed 48 hours after the initial dose of T.S.H.
[2] Mean difference in P.B.I. level, +2·6 μg per 100 ml.

Table V shows the response in I^{131} uptake following T.S.H. administration to a group of four patients with clinically confirmed primary hypothyroidism. Here the increase in I^{131} uptake ranged from 0·7% to 3·2%, with a mean value of +1·8%.

TABLE IV

The Effect of T.S.H. on I^{131} Uptake of Seven Clinically Euthyroid Patients in Whom the Diagnosis of Hypothyroidism had been Suspected on Clinical Grounds Months or Years Prior to These Investigations.[1]

Patient	I^{131} Uptake (Percentage)[2]			Remarks
	Before T.S.H.	After T.S.H.	Difference	
A.C. ..	3·2	37·4	+34·2	? Hypothyroid; receiving thyroid
L.D. ..	10·5	38·7	+28·2	? Hypothyroid; receiving thyroid
J.K. ..	8·1	37·6	+29·5	? Hypothyroid; receiving thyroid
D.B. ..	9·0	24·7	+15·7	? Hypothyroid; off thyroid therapy for six weeks
F.J. ..	2·0	28·7	+26·7	? Hypothyroid; off thyroid therapy for two weeks
R.B.	12·1	58·4	+46·3	? Hypothyroid; off thyroid therapy for six weeks
L.M. ..	9·4	33·6	+24·2	? Hypothyroid; off thyroid therapy for four weeks

[1] Table I includes four of these patients.
[2] Mean difference in I^{131} uptake, +29·3%.

In Table VI is shown the increase in I^{131} uptake in five cases of clinically confirmed hypopituitarism. The rise in uptake ranged from 20·4% to 35·5%, the mean being +24·5%.

These results show a clear difference between the euthyroid and primary myxœdema groups, with a much smaller increase in uptake in the latter group. However, those patients suffering from hypothyroidism consequent upon hypopituitarism gave responses which were similar to the euthyroid group.

T_3-Induced Changes in I^{131} Uptake in Euthyroidism and Hyperthyroidism

In Table VII are recorded the findings in 11 euthyroid patients. Four of these were normal controls with an initial I^{131} uptake ranging

TABLE V

The Effect of T.S.H. on I^{131} Uptake of Four Patients with Hypothyroidism. Two Patients were Receiving Thyroid Medication

Patient	I^{131} Uptake (Percentage)[1]			Remarks
	Before T.S.H.	After T.S.H.	Difference	
K.S. ..	1·6	3·9	+2·3	Cretinism; receiving thyroid
D.P. ..	4·4	5·1	+0·7	Myxœdema; receiving thyroid
E.S. ..	2·1	3·2	+1·1	Juvenile myxœdema
M.S. ..	4·0	7·2	+3·2	Myxœdema

[1] Mean difference in I^{131} uptake, +1·8%.

from 13·9% to 32%, while the remaining seven, with uptakes ranging from 46·1% to 83·8%, were originally suspected to be thyrotoxic. However, the P.B.I. in all cases was within normal limits. After the administration of T_3, the I^{131} uptake was suppressed in all patients.

TABLE VI

The Effect of T.S.H. on the I^{131} Uptake of Five Patients with Hypopituitarism

Patient	I^{131} Uptake (Percentage)[1]			Remarks
	Before T.S.H.	After T.S.H.	Difference	
J.O'S.	18·5	54·0	+35·5	Hypopituitarism, ætiology obscure
H.D. ..	6·2	27·1	+20·9	Hypopituitarism, ætiology obscure
E.K. ..	14·7	38·2	+23·5	Sheehan's syndrome
M.D. ..	11·4	31·8	+20·4	Sheehan's syndrome
M.S. ..	18·9	41·2	+22·3	Sheehan's syndrome

[1] Mean difference in I^{131} uptake, +24·5%.

In the doubtfully toxic group the magnitude of suppression was such that I^{131} uptake now fell to within the euthyroid range—in one patient (G.R.) into the range of hypothyroidism.

In Table VIII the results of I^{131} uptake before and after T_3 administration in five cases of confirmed thyrotoxicosis are presented. In no patient was suppression of I^{131} uptake observed—on the contrary, it rose. This is especially

TABLE VII

The Effect of T_3 Administration on the I^{131} Uptake and P.B.I. Levels of 12 Euthyroid Patients (Four Normal Controls and Eight with Initial High I^{131} Uptake)[1]

Patient	I^{131} Uptake (Percentage)[2]			P.B.I. Level (Microgrammes per 100 ml.)[3]			Remarks
	Before T_3	After T_3	Difference	Before T_3	After T_3	Difference	
B.H. ..	25·4	12·2	−13·2	4·6	3·2	−1·4	Normal control
J.C. ..	13·9	1·3	−12·6	4·3	3·0	−1·3	Normal control
W.E. ..	32·0	1·3	−30·7	4·8	2·8	−2·0	Normal control
McK.D. ..	18·5	4·1	−14·4	2·9	2·9	±0·0	Normal control
J.P. ..	62·7	10·2	−52·5	4·7	2·9	−1·8	? Thyrotoxicosis; P.B.I.^{131} level normal
K.K. ..	74·2	17·8	−56·4	4·0	6·5	+1·8	Goitre; previous medication with neomercazole; P.B.I.^{131} level normal
D.McD. ..	59·3	9·2	−50·1	4·2	1·9	−2·3	Goitre; tachycardia; P.B.I.^{131} level normal
N.S. ..	62·8	22·9	−39·9	5·2	2·6	−2·6	Goitre, non-toxic; P.B.I.^{131} level normal
G.R. ..	83·8	7·2	−77·6	4·0	1·4	−2·6	Goitre, ? toxic; P.B.I.^{131} level normal
B.S. ..	55·7	26·7	−29·0	4·9	3·0	−1·9	Goitre; tachycardia; ? thyrotoxicosis
M.L. ..	62·7	39·6	−23·1	3·4	2·5	−0·9	Goitre, ? toxic; P.B.I.^{131} level, thyrotoxicosis
H.E. ..	46·1	13·6	−32·5	5·7	4·2	−1·5	? Toxic; ? thyrotoxicosis; P.B.I.^{131} level, thyrotoxicosis

[1] P.B.I.^{131} refers to the measurement of radioactive P.B.I. 48 hours after tracer dose of I^{131}—Clarke, Sherriff and Winikoff, 1955.
[2] Mean difference in I^{131} uptake: normal controls, −17·7%; euthyroid patients, −45·1%.
[3] Mean difference in P.B.I. level: normal controls, −1·2 μg. per 100 ml.; euthyroid patients, −1·9 μg. per 100 ml.

significant in the case of R.T., in which the initial uptake indicated euthyroidism despite the clinical diagnosis of thyrotoxicosis and a P.B.I. level of 12·5 μg. per 100 ml.

T_3-Induced Changes in P.B.I. Levels in Euthyroidism and in Hyperthyroidism

In Tables VII and VIII are also recorded the P.B.I. levels before and after T_3 administration to euthyroid subjects (including four normal controls) and to thyrotoxic patients respectively.

In the former group (Table VII), a fall in P.B.I. level was observed in all but two cases. In one of the normal controls (McK.D.) no change occurred, and in the other (K.K.) a rise of 1·8 μg. per 100 ml. was noted.

In the thyrotoxic group (Table VIII), all P.B.I. levels were depressed by T_3. No characteristic degree or pattern of response was noted, which invalidates T_3-induced changes in P.B.I. levels as a means of distinguishing euthyroidism from hyperthyroidism. This is not surprising, since T_3 will not suppress I^{131} uptake in hyperthyroidism, but will diminish thyroid hormone production.

DISCUSSION

The results of this study show that the use of T.S.H. and T_3 as adjuvants to routine laboratory procedures can be of assistance in the differential diagnosis of obscure cases of thyroid disease. They are summarized in Table IX.

The use of T.S.H. and its effect on thyroid function have been described by Sharpey-Schafer and Schrire (1939), by Stanley and Astwood (1949), by Querido and Stanbury (1950), by Levy *et alii* (1953), by Schneeberg *et alii* (1954),

TABLE VIII

The Effect of T_3 Administration on the I^{131} Uptake and P.B.I. Level in Five Hyperthyroid Patients[1]

Patient	I^{131} Uptake (Percentage)[2]			P.B.I. Level (Microgrammes per 100 ml.)[3]			Remarks
	Before T_3	After T_3	Difference	Before T_3	After T_3	Difference	
A.T. ..	82·7	88·5	+ 5·8	14·3	11·0	−3·3	Clinical thyrotoxicosis; P.B.I.^{131} level, thyrotoxicosis
B.C. ..	83·5	86·4	+ 2·9	8·0	6·0	−2·0	Clinical thyrotoxicosis; P.B.I.^{131} level, thyrotoxicosis
S.D. ..	64·9	95·1	+30·2	10·2	8·7	−1·5	Clinical thyrotoxicosis; P.B.I.^{131} level, thyrotoxicosis
R.T. ..	27·5	47·0	+19·5	12·5	7·1	−5·4	Clinical thyrotoxicosis; P.B.I.^{131} level, high normal.
E.H. ..	56·8	59·6	+ 2·8	8·0	6·0	−2·0	Clinically, ? thyrotoxicosis; P.B.I.^{131} level, borderline thyrotoxicosis

[1] The first three patients, clearly toxæmic, served as controls.
[2] Mean difference in I^{131} uptake, +12·2%.
[3] Mean difference in P.B.I. level, −2·8 μg. per 100 ml.

TABLE IX

The Response to T.S.H. and T_3 Administration Measured by Plasma P.B.I. Level and Thyroidal I^{131} Uptake in Euthyroidism, in Primary and Secondary Hypothyroidism and in Hyperthyroidism

Nature of Stimulus	Group of Disorders	Difference in P.B.I. Level (Microgrammes per 100 ml.)	Change in I^{131} Uptake (Percentage)	Tables
Administration of T.S.H. (four one-millilitre capsules of "Ambinon B" every 12 hours)	(*a*) Normal function (controls); (*b*) normal function (? hypothyroidism)	+5·3 (11)	+29·3 (7)	I, IV
	Hypothyroidism (primary)	+0·7 (11)	+ 1·8 (4)	II, V
	Hypothyroidism (secondary)	+2·6 (11)	+24·5 (5)	III
Triiodothyronine (25 μg. three times a day for seven to ten days)	(*a*) Normal function (controls); (*b*) normal function (? hyperthyroidism)	−1·7 (12)	−37·7 (12)	VII
	Hyperthyroidism	−2·8 (5)	+12·2 (5)	VIII

by Friss *et alii* (1959), and others. These results include elevation of the basal metabolic rate, and rises in I^{131} uptake and in P.B.I. levels. Our results confirm the two last-mentioned effects. These have been found the most sensitive by previous workers.

The studies on normal patients reported here show, in response to the injection of T.S.H., a substantial increase in the four-hour uptake of I^{131} by the thyroid and an elevation in P.B.I. concentration. These tests have been valuable in excluding primary myxœdema in patients whose condition had been incorrectly diagnosed, and who, at the time of investigation, were being unnecessarily treated with thyroid extract. In these cases the elevation in both indices showed the responsiveness of the gland to stimulation by T.S.H., indicating essentially normal thyroid function. It should be noted that the initial levels of P.B.I. and I^{131} uptake (when elevated and depressed respectively by exogenous thyroid) can be misleading.

In myxœdema it is important to determine the site of thyroid failure, since replacement therapy differs in primary and secondary hypothyroidism. The clinical differentiation may be confused by the depression of adrenal and gonadal function sometimes observed in long-standing primary myxœdema (Hill *et alii*, 1950; Garrod and Gilliland, 1954).

The magnitude of the response of P.B.I. level and I^{131} uptake to T.S.H. administration, being negligible in primary myxœdema (Tables II and V), and intermediate between this and normal in patients with secondary (pituitary) myxœdema (Tables III and VI), clearly differentiates between these two groups. However, it is to be noted that in two patients with pituitary myxœdema a very small rise in P.B.I. levels was observed. One of these (J.T.) presented the classical picture of Sheehan's syndrome, with a long history and the subsequent occurrence of secondary atrophy and fibrosis of the thyroid gland—a condition described by Sheehan and Summers (1949), by Querido and Stanbury (1950), and by Garrod and Gilliland (1954). The other (J.D.) had had an ependymoma removed seven years earlier, the operation being followed by massive irradiation of the pituitary area. Since the initial P.B.I. level in this case was not very low, the small response could be explained by the fact that the thyroid was producing hormone to its maximum capacity and therefore incapable of responding to further stimulation.

These circumstances—secondary fibrosis and low thyroid reserve—place limitations on these methods of investigation.

The administration of thyroid hormone has a profound influence on thyroid gland activity, as described by Farquharson and Squires (1941) and by Riggs *et alii* (1945). Greer (1951) noted that the uptake of I^{131} by the gland was depressed by thyroid medication. In our study, this effect of administered thyroid depressing I^{131} uptake was noted as long as six weeks after the cessation of therapy (Table IV) in patients shown by other means to be euthyroid.

The significance of an elevated I^{131} uptake in the diagnosis of thyrotoxicosis has been subjected to criticism, since certain doubtfully hyperthyroid patients, often with goitre and despite values within the "toxic" range, have been proven subsequently to be euthyroid or even hypothyroid. These discrepancies have been observed by two of us (Hudson and Martin, 1959).

The mechanism increasing the avidity of the gland for iodine is incompletely understood. Iodine deficiency has been postulated by some authors. The rebound phenomenon with high I^{131} uptake following withdrawal of antithyroid drugs has been studied by Greer and Smith (1954) and by Burrell and Fraser (1957). In most cases no explanation can be offered.

A difficult diagnostic problem is presented by that group of patients in whom thyrotoxicosis is still suspected after treatment by surgery or I^{131} (Martin and Stanbury, 1955; Hudson and Martin, 1959). There is often an elevated I^{131} uptake and a raised forty-eight-hour P.B.I.^{131} level associated with a normal or low P.B.I. level. It is thought that the explanation of these paradoxical findings lies in the rapid turnover of the hormone produced by a small thyroid remnant and its prompt peripheral utilization.

In the suspect thyrotoxic with high I^{131} uptake, the exhibition of thyroid hormone produces a substantial suppression in this parameter of assessment of thyroid function while the gland is, in fact, normal. In thyrotoxicosis, on the other hand, no such suppression occurs. The use of thyroid hormone, administered exogenously as desiccated thyroid, thyroxine or T_3, in the differentiation between thyrotoxicosis and those euthyroid states with high I^{131} uptake, has been described by Morgans *et alii* (1952), by Greer and Smith (1954), and by Werner and Spooner (1955). Our results show clear differences in I^{131} uptake after T_3 administration in these two groups of patients (Tables VII and VIII). As was mentioned earlier, the P.B.I. measurements did not contribute to the differential diagnosis, particularly in the thyrotoxic group.

From our experiences, we conclude that the T.S.H. and T_3 tests, as we have applied them, offer further means of diagnosis of both myxœdema and thyrotoxicosis. They have their place in a small number of cases in which the differential diagnosis by routine methods is inadequate.

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CHANGES IN URINARY STEROID EXCRETION DURING EXAMINATIONS

ROGER MELICK[2]

From the University of Melbourne Department of Medicine in the Royal Melbourne Hospital

SUMMARY

Urinary total 17-hydroxycorticosteroid (17-OHCS) excretion and 17-ketosteroid (17-KS) excretion by 10 university students during day and night periods were measured four weeks before and during university annual examinations. A significant increase in the excretion of total 17-OHCS occurred during examinations in both day and night periods. No rise occurred in 17-KS excretion. The normal diurnal variation of excretion was present both before and during examinations for both total 17-OHCS and 17-KS. It is concluded that examinations caused an increase in basal total 17-OHCS excretion as well as an increase during the examinations themselves, and that the adrenal-stimulating factor was not enough to cause a rise in 17-KS excretion detectable by the method used.

VARIOUS emotional experiences are capable of increasing blood levels and urinary excretion of adrenal cortical steroids. The plasma level of 17-hydroxycorticosteroids (17-OHCS)[3] rises during emotional upsets (Bliss *et alii*, 1956; Mason, 1959), before surgery (Franksson and Gemzell, 1955), after surgery (Sandberg *et alii*, 1954), and after supersonic flight (Hale *et alii*, 1959). Urinary 17-OHCS excretion rises during emotional upsets (Hetzel *et alii*, 1955), competitive rowing (Hill *et alii*, 1956), and examinations (Bliss *et alii*, 1956). Mason (1959) has described rises in urinary 17-OHCS excretion in patients on the day prior to surgery, in soldiers on the day before a 96-hour sleep-deprivation exercise, and in crew members of a jet bomber during an 11,000 mile non-stop flight. Examinations have also caused a rise in the excretion of formaldehydogenic steroids (Schwartz and Shields, 1956), of 17-ketogenic steroids (Connell *et alii*, 1958), and of aldosterone (Venning *et alii*, 1957). Urinary excretion of 17-ketosteroids (17-KS) rose under the stress of flying (Pincus and Hoagland, 1943) and immediately before competitive rowing (Hill *et alii*, 1956), but did not rise significantly during examinations (Connell *et alii*, 1958).

Mason (1959), Connell *et alii* (1958) and Hill *et alii* (1956) collected urine samples during the whole 24-hour period; other investigators have concentrated on short collection periods embracing the incident causing stress. In normal people, the excretion of steroid fluctuates, being highest early in the morning and falling progressively to the lowest level in the evening and night. Hill *et alii* showed that the stress of competitive rowing at 5 p.m. tended to flatten out this diurnal variation; this may have been due to the timing of the race, but serves to draw attention to alterations in steroid excretion throughout the day rather than just in the few hours embracing the incident causing stress.

This study was designed to determine whether the rise in urinary corticoid excretion produced by examinations was paralleled by a rise in 17-KS excretion, and to observe the effect of examination stress upon the night excretion of both groups of steroids.

MATERIAL AND METHODS

The subjects were 10 male university students aged from 17 to 34 years; the mean age was 25·1 years. All were resident in a university college, and all were studying for university annual examinations. All examinations were written papers of either two or three hours' duration.

Two sets of urine samples were obtained, the preexamination sample and the examination sample; both sets were collected as "night" and "day" samples. Night collections were made from 8 p.m. to 8 a.m. and day collections

[1] Received on March 7, 1960.

[2] Assistant in the University Department of Medicine.

[3] In this paper 17-hydroxycorticosteroids (17-OHCS) refer to those substances measured by the Porter-Silber reaction (17 : 21-dihydroxy-20-keto-corticosteroids — Porter and Silber, 1950); 17-ketogenic steroids are those measured by the method of Norymberski *et alii* (1953) and include 17 : 21-dihydroxy-20-corticosteroids, 17 : 20 : 21-triols and 17-20-glycols. Total 17-OHCS refer to the substances measured by the method of Appleby *et alii* (1955); these include the ketogenic steroids and 17-hydroxy-20-keto-corticosteroids. The term "corticoid" refers to (urinary) cortisol metabolites in general.

from 8 a.m. to 8 p.m. The maximum deviation from these times for both sets was 30 minutes. In both preexamination and examination periods, the subjects retired between 11.30 p.m. and 1 a.m., and rose between 7 a.m. and 8 a.m.

The preexamination samples were collected from three to four weeks before the first examination. Three night and three day samples were obtained from each of nine subjects, and two of each from the tenth.

The examination samples commenced with a night collection on the night preceding the first examination and a day collection on the day of the first examination. Similar samples were obtained on the next day on which the subjects had an examination. Thus two night and two day samples were obtained from all 10 subjects.

The examinations were on successive days for some subjects, but not all. Some examinations were in the morning, and some in the afternoon. Some subjects had two examinations on one or both of the examination days.

TABLE I

Urinary 17-Ketosteroid Excretion

Subject	Night		Day	
	Pre-examination (mg./hr.)	Examination (mg./hr.)	Pre-examination (mg./hr.)	Examination (mg./hr.)
1	0·40	0·39	0·55	0·61
2	0·44	0·35	0·75	0·69
3	0·35	0·41	0·53	0·61
4	0·38	0·44	0·53	0·59
5	0·60	0·78	0·71	0·77
6	0·58	0·33	0·66	0·50
7	0·47	0·46	0·62	0·78
8	0·47	0·40	0·48	0·55
9	0·90	0·75	1·36	1·22
10	0·48	0·55	0·54	0·51
	Mean difference, −0·022; t, 0·556; $P>0·5$		Mean difference, +0·010 t, 0·345; $P>0·6$	

Samples were collected without preservative, and aliquots were frozen within 12 hours of completion of collection until they were analysed. On all samples, 17-KS content was determined by the method of Vestergaard (1951), and total 17-OHCS content by a modification of the method of Appleby *et alii* (1955). The significance of the difference between the various groups was determined by the paired t test.

RESULTS

The results for similar periods have been grouped together, and all results expressed as milligrammes of steroid excreted per hour.

There was no change in 17-KS excretion from preexamination to examination periods. The mean difference was −0·02 mg. per hour for the night excretion, and +0·01 mg. per hour for the day excretion (Table I and Figure I). Neither change is significant.

The total 17-OHCS excretion in the examination period was higher than in the preexamination period, both night and day. The mean

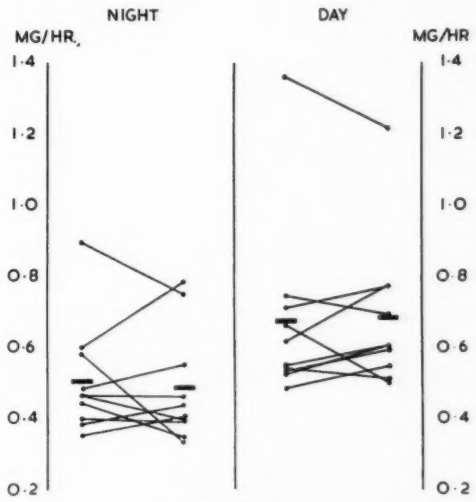

FIGURE I

Change in 17-ketosteroid excretion, both by night and by day, before and during examinations. Preexamination values are represented by the dots at the left end of the lines, and examination values by the dots at the right end. Means are shown by the horizontal bars

difference for night excretion was +0·12 mg. per hour ($P<0·05$); the excretion rose in seven subjects and fell in three. The mean difference for day excretion was +0·24 mg. per hour ($P<0·01$); the excretion rose in nine subjects and fell in one (Table II and Figure II).

The excretion of both 17-KS and total 17-OHCS during the day was significantly greater than the excretion during the night in both control and experimental periods (Table III).

DISCUSSION

The rise in urinary excretion of total 17-OHCS in the examination period is consistent with the reports cited earlier. This rise was observed in both day and night samples. Most of the reports have not commented on night excretion. However, Hill *et alii* (1956) noted raised urine 17-OHCS excretion from 9 p.m. till 9 a.m. after competitive rowing, while Connell *et alii* (1958)

observed a slight increase in urinary 17-ketogenic steroid excretion from 11 p.m. till 8.30 a.m.; they did not comment upon this, and apparently regarded it as too small to be significant.

TABLE II

Urinary Total 17-Hydroxycorticosteroid Excretion

Subject	Night		Day	
	Pre-examination (mg./hr.)	Examination (mg./hr.)	Pre-examination (mg./hr.)	Examination (mg./hr.)
1	0·29	0·52	0·46	0·83
2	0·33	0·48	0·74	1·02
3	0·31	0·42	0·38	0·69
4	0·42	0·52	0·59	0·96
5	0·76	0·71	0·93	1·15
6	0·41	0·77	0·64	0·95
7	0·39	0·37	0·60	0·82
8	0·48	0·32	0·43	0·77
9	0·52	0·69	1·08	0·84
10	0·27	0·57	0·22	0·47
	Mean difference, +0·117; *t*, 2·33; *P*<0·05		Mean difference, +0·243; *t*, 4·35; *P*<0·01	

The increase in night excretion is very interesting, and raises the question whether emotional stress can cause increased adrenal secretion during sleep. The night collection

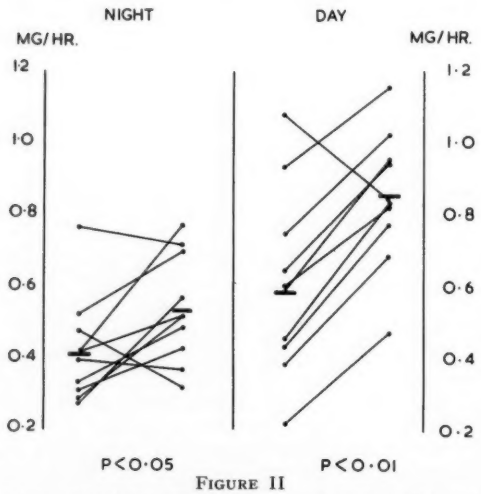

FIGURE II

Change in total 17-OHCS excretion, both by night and by day, before and during examinations. Preexamination values are represented by the dots at the left end of the lines, and examination values by the dots at the right end. Means are shown by the horizontal bars

period here covered the night before examination, and the subjects were awake for about five hours and asleep for about seven. It is possible that all the night increase was during waking (and studying) hours, and that steroid excretion during sleep was not altered. If one assumes the excretion during the five waking hours (8 p.m. to 1 a.m.) to be the same as the average day excretion, then the excretion during sleep is not significantly raised (mean difference +0·025 mg. per hour). If the normal diurnal pattern of steroid excretion is present, this calculation is not correct, as the lowest waking excretion occurs from 8 p.m. to midnight. It is possible that the stress of examinations altered this pattern, as in Hill's rowers. However, this is unlikely, as the normal pattern of greater day than night excretion was present both before and during examination. In addition, Mason (1959) found that the diurnal variation of plasma 17-OHCS level was not altered in soldiers facing a sleep-deprivation

TABLE III

Comparison of Night and Day Excretion

Samples	Mean Difference (mg./hr.)	*t*	*P*
17-KS, preexamination ..	+0·166	4·036	<0·01
17-KS, examination	+0·199	4·057	<0·01
Total 17-OHCS, preexamination	+0·189	3·180	<0·02
Total 17-OHCS, examination ..	+0·314	5·124	<0·001

exercise, despite significantly raised plasma levels of 17-OHCS at 8 a.m. This study strongly suggests that there was an increase in steroid excretion throughout the whole 24 hours of the examination days.

In contrast to the excretion of total 17-OHCS, 17-KS excretion did not alter, either by day or by night. This finding confirms that of Connell *et alii* (1958), but differs from that of Hill *et alii* (1956). Emotional factors cause increased release of ACTH from the pituitary (Harris, 1955). Exogenous ACTH given in a dosage of 20 to 80 units intravenously or intramuscularly, causes a rise in both 17-KS and corticoid excretion, but the rise in 17-KS excretion is generally much less than the rise in corticoid excretion both in our laboratory and in others (Felber *et alii*, 1959; Heider *et alii*, 1959). The rise in total 17-OHCS excretion observed in the examination period is slight compared with that obtained from the amount of exogenous ACTH usually given. Probably either the extra amount of ACTH released by the examination stress was not enough to increase 17-KS excretion, or the method of analysis used was not sensitive enough to detect a small rise. It is possible that the adrenal-stimulating factor acted only on 17-OHCS excretion and did not affect 17-KS excretion; but this is unlikely, as increases in 17-KS excretion during

stress have been cited previously, showing that, at least in some instances, the adrenal-stimulating factor can affect 17-KS excretion. The explanation favoured here is that the increase in adrenal stimulation was not enough to cause an increase of 17-KS excretion detectable by a standard clinical method.

ACKNOWLEDGEMENTS

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VENO–ARTERIAL SHUNTING OF BLOOD IN CHRONIC LIVER DISEASE[1]

C. R. B. Blackburn,[2] John Read,[3] J. McRae,[4] H. J. Colebatch,[5] M. R. Playoust[4] and R. A. B. Holland[5]

From the Department of Medicine, University of Sydney, and the Clinical Research Unit, Royal Prince Alfred Hospital, Sydney[6]

Summary

Seventeen out of 43 patients with chronic liver disease had arterial oxygen unsaturation at rest breathing room air. This incidence is higher than has been recognized in the past. The degree and basis of this arterial oxygen unsaturation were studied further in 14 patients, of whom seven had arterial oxygen unsaturation at rest. An alveolar-capillary diffusion defect played some part in its production in one patient, and the ventilation-perfusion inequality of chronic airway disease was partly or wholly responsible in five others.

After allowance had been made for respiratory factors, there remained at least six, and possibly 10, patients out of 14 in whom an abnormal degree of veno-arterial shunting of blood could be demonstrated. The dimensions of this shunt ranged up to more than 20% of the cardiac output. On theoretical grounds, it is likely that a significant part of the veno-arterial shunting is intrapulmonary.

For many years, cyanosis, finger clubbing and arterial oxygen unsaturation have been recognized as occurring in patients with cirrhosis of the liver, but they are not regarded as common. An obvious cause for these phenomena is found in some patients who have coexistent chronic pulmonary disease. Indeed, the presence of chronic bronchitis and emphysema in the alcoholic cirrhotic should occasion no great surprise. Several anatomical studies have provided evidence that there may be portal vein-left auricular shunting and pulmonary arterio-venous shunting of blood in patients with cirrhosis of the liver (Calabresi and Abelmann, 1957; Hales, 1956; Rydell and Hoffbauer, 1956). Physiological studies indicating venous admixture have been carried out by Wilson *et alii* (1953) and by Murray *et alii* (1958), and in some of the reported cases the shunts appear to be large.

The present paper reports studies carried out to determine the incidence of arterial oxygen unsaturation among patients with chronic liver disease, and to determine the part played by veno-arterial shunting of blood and chronic respiratory disease in its genesis.

[1] Received on March 11, 1960.
[2] Professor of Medicine.
[3] Senior Lecturer in Medicine.
[4] Fellows in Medicine, Royal Prince Alfred Hospital.
[5] Research Fellows, Department of Medicine.
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Materials and Methods

Forty-three patients with chronic liver disease (progressive hepatitis, cirrhosis, hæmochromatosis) were studied.

Study A

Arterial blood samples were collected anaerobically by percutaneous brachial artery puncture from all 43 patients. The oxygen saturation of the arterial blood was determined by the method of Van Slyke and Neill (1924) and corrected for dissolved oxygen.

Study B

Fourteen patients were studied more completely, three of whom were chosen specifically because they had clinical cyanosis. The remainder consisted of unselected subjects from among those patients with chronic liver disease admitted to the Clinical Research Ward of the Royal Prince Alfred Hospital during 1957, 1958 and 1959. The resting arterial oxygen saturations of these 14 patients are included among those of the 43 patients referred to in Study A.

The patients rested quietly in the laboratory two hours after breakfast, having abstained from smoking on the morning of the tests. A Riley needle was inserted into the brachial artery after local tissue infiltration with lignocaine. Gas expired over a measured time was collected into Douglas bags during the various experimental procedures, and simultaneous arterial blood

samples were collected anaerobically in paraffined syringes. The syringes were kept in melting ice until the analysis was performed.

Specimens of arterial blood and expired gas were collected: (i) while the patient breathed room air, at rest, semi-recumbent; (ii) while the patient breathed 100% oxygen, at rest, semi-recumbent, after 10 minutes had been allowed for equilibrium to be reached; (iii) while the patient exercised on a bicycle ergometer breathing room air; (iv) while the patient exercised on a bicycle ergometer breathing 100% oxygen.

Three minutes were allowed to reach a steady state in the two last-mentioned procedures. From one patient, who was unable to exercise because of the effects of peripheral neuropathy, an arterial blood sample was collected during vigorous hyperventilation with 100% oxygen instead of during exercise.

Ventilatory capacity was assessed in 12 patients by determination of the vital capacity and the maximum breathing capacity. The latter was measured directly in four patients, and calculated from the one-second forced expiratory volume by multiplying by a factor of 35 in eight patients. In the latter eight patients, the ratio of one-second forced expiratory volume to vital capacity ($F.E.V._{\cdot 1\cdot 0}/V.C.$ ratio—Gandevia and Hugh-Jones, 1957) was used as an additional index of airway disease.

The diffusing capacity of the lungs for carbon monoxide (D_{CO}) was determined in five patients by the steady-state method of Filley *et alii* (1954) as modified in this laboratory (Holland and Blacket, 1958).

All arterial blood oxygen saturations were determined by the method of Van Slyke and Neill (1924) and corrected for dissolved oxygen. Arterial blood carbon-dioxide content was determined by the method of Van Slyke and Neill (1924) and the arterial blood pH with a Stadie anaerobic cell and a Cambridge pH meter, and arterial carbon dioxide tension was read from the nomogram of Singer and Hastings (1948).

Expired gas was analysed by the classical Haldane technique.

TABLE I

Arterial Oxygen Saturation (Percentage)	Number of Patients
<90	4
90–91	1
91–92	2
92–93	3
93–94	4
94–94·2	3
>94·3	26
Total	43

RESULTS

Study A

The lower limit of arterial oxygen saturation (Corrected) in this laboratory is 94·3% in normal subjects at rest breathing room air. Of the 43 subjects presently studied, 17 had resting arterial oxygen saturation below this figure. The distribution of the arterial oxygen saturation is shown in Table I.

Study B

The pertinent results are shown in Table II. Of the 14 patients, seven had resting arterial oxygen saturations below the normal range

TABLE II

Case Number	Patient's Age (Years)	Arterial Oxygen Saturation (Percentage)				Exercise Oxygen Consumption (ml./M²/min.)	Vital Capacity (Litres)	M.B.C.[1] (L per min.)	$F.E.V._{\cdot 1\cdot 0}/V.C.\%$[2]	V_D/V_T Ratio (Rest)[3]	D_{CO}[4]
		Rest, Air	Rest, Oxygen	Exercise, Air	Exercise, Oxygen						
1	48	91·5	97·5	76·2	91·2	430	4·5	121	—	0·35	8·3
2	58	96·8	96·4	94·8	93·3	430	2·9	84	84	0·29	—
3	42	89·7	97·8	—	94·5	—	—	—	—	0·36	18·3
4	54	93·3	95·5	—	95·9	—	4·2	96	65	—	—
5	35	95·7	97·2	94·2	96·0	727	3·2	109	—	0·28	17·2
6	52	91·4	96·4	—	96·2[5]	—	3·4	63	53	0·40	—
7	45	93·1	97·1	95·6	97·1	273	4·1	96	68	0·47	—
8	42	95·9	98·8	96·2	97·3	274	2·4	63	74	0·27	—
9	59	83·9	93·3	90·5	97·4	353	2·6	61	68	0·36	—
10	13	99·3	98·9	97·6	97·8	—	3·2	118	—	0·25	—
11	51	92·2	96·6	94·9	98·0	581	3·1	64	63	0·47	—
12	17	95·8	98·3	94·0	98·5	—	—	—	—	0·31	20·0
13	42	95·9	97·5	97·5	98·7	568	3·3	122	—	0·38	18·1
14	55	95·8	99·5	—	100·9	207	2·6	65	71	0·25	—

[1] M.B.C.: Maximum breathing capacity.
[2] $F.E.V._{\cdot 1\cdot 0}/V.C.\%$: Percentage of vital capacity expired in first second of a forced expiration.
[3] V_D/V_T ratio: Ratio of dead space to tidal volume.
[4] D_{CO}: Diffusing capacity for carbon monoxide in millilitres per mm tension gradient per minute.
[5] Hyperventilation value.

breathing room air; that is, the incidence of desaturation was similar to that in the larger series of 43. Resting arterial oxygen saturation rose in 12 subjects when breathing pure oxygen, but remained below 98% in 10. In no case did the arterial oxygen saturation fall when the subject was breathing 100% oxygen, allowance being made for a Van Slyke error of up to 0·5% saturation. The subject's response to exercise while breathing air varied, and probably depended on variable redistribution of ventilation-perfusion inequalities. Exercise on pure oxygen led to the series of arterial oxygen saturations ranging from 91·2% to 100·9%, by which the subjects are ranked in Table II. The degree of veno-arterial shunting that this represents is shown in Table III. That exercise was relatively light is shown by the oxygen consumption figures during exercise. When arterial oxygen saturations in subjects breathing oxygen at rest and on exercise are compared, it is seen that exercise led to a significant fall in saturation in six (Cases 1, 2, 3, 5, 8, 10), to no significant change in five (Cases 4, 6, 7, 12, 14), and to a rise in three (Cases 9, 11, 13).

TABLE III

Veno-Arterial Shunt as Percentage of Cardiac Output[1] *on Exercise during Breathing of Pure Oxygen*

Case Number	If A-V Oxygen Difference is Assumed to be (Volumes per Centum)				
	4	5	6	7	8
1	32·1[2]	27·4[2]	24·0[2]	21·3[2]	19·1[2]
2	24·7	20·8	17·9	15·8	14·1
3	18·2	15·1	12·9	11·3	10·0
4	14·5	12·0	10·2	8·9	7·8
5	18·4	15·2	13·0	11·4	10·1
6	14·2	11·7	9·9	8·6	7·6
7	9·7	7·9	6·7	5·8	5·1
8	11·1	9·1	7·7	6·7	5·9
9	12·3	10·1	8·5	7·4	6·5
11	6·3	5·1	4·3	3·7	3·3
12	6·5	5·3	4·5	3·8	3·4
14	0	0	0	0	0

[1] The shunt dimensions do not necessarily run parallel with the arterial oxygen saturations, because the shunt equation depends on total oxygen contents, which will be influenced by hæmoglobin concentration.

[2] These values would only be a little reduced if one allowed a large end-capillary oxygen gradient because of the diffusion defect; for example, allowing a large end-capillary gradient for oxygen of 100 mm. of mercury still leaves a shunt of 29% at an arterio-venous oxygen difference of 4.

The remaining tests of respiratory function indicated (*a*) a significant diffusion defect in Case 1 (D_{CO}=8·3 ml. CO/mm. Hg), and (*b*) some degree of airway disease and/or ventilation-perfusion inequality in Cases 4, 6, 7, 9 and 11 (shown by one or more of the following: low maximum breathing capacity, low $F.E.V._{1\cdot0}/V.C.$ ratio, high V_D/V_T ratio). No patient in the group had a raised arterial carbon-dioxide tension.

DISCUSSION

The incidence of arterial oxygen unsaturation at rest among patients in the present series is high—nearly 40%—compared with less than 20% in the slightly smaller series of Murray *et alii* (1958).

Arterial oxygen unsaturation while the subject is breathing room air may be due to alveolar hypoventilation, to an alveolar-capillary diffusion defect, to ventilation-perfusion inequalities, or to veno-arterial shunting of blood. Breathing 100% oxygen restores arterial oxygen saturation to normal in cases of hypoventilation or diffusion defect, and in most cases of ventilation-perfusion inequality. Hyperventilation with 100% oxygen (either voluntary, or on exercise) restores normal saturation in the remaining cases of ventilation-perfusion inequality (Colebatch and Read, unpublished observations). Significant arterial oxygen unsaturation persisting after these measures must be due to shunting of blood from the venous to the arterial side of the circulation. Strictly, then, one need not define the status of respiratory function among patients in whom a shunt is sought by these means. On the other hand, the results are lent added validity if one is able to establish the part played by respiratory malfunction in the arterial oxygen desaturation frequently demonstrated at rest.

The data of Table II show that almost all subjects had a significant rise in arterial oxygen saturation when breathing pure oxygen. This is consistent with reduction of the effect of a small ventilation-perfusion inequality among those who seemed to have normal respiratory function (Read, 1959), and of a larger ventilation-perfusion inequality in those patients with airway disease (Cases 4, 6, 7, 9, 11). Likewise the diffusion defect in Case 1 would be overcome.

That a number of patients still had arterial oxygen unsaturation when exercising on oxygen (Table II) indicates that they had significant veno-arterial shunting of blood. The dimensions of this shunt cannot be stated precisely unless the arterio-venous oxygen difference is known, and cardiac catheterization was not performed in the present studies. However, the order of magnitude of the shunts can be appreciated from Table III, where the shunts corresponding to a series of possible arterio-venous oxygen differences are given. It should be noted in this context that the cardiac output is frequently high in patients with cirrhosis (Abelmann and Kowalski, 1953), and the exercise was only light to moderate. Arterio-venous oxygen differences are thus likely to lie among the lower values

assumed, and the veno-arterial shunts towards the upper calculated values.

The calculated amount of blood shunted ranged from none up to more than 20% of the cardiac output. The higher values are obviously abnormal, and we prefer to state the remainder as a continuous series rather than to attempt to define a strict upper limit of normality. This has been placed at 7% of the cardiac output of a subject at rest breathing room air (Comroe *et alii*, 1955). Certainly, in several patients in this series both with (Case 11) and without (Cases 12 and 14) respiratory disease, the calculated shunt was very small.

The presence of arterial oxygen unsaturation in patients with chronic liver disease was first reported by Snell (1935). That this could be due to abnormal veno-arterial shunting of blood was shown functionally by Wilson *et alii* (1953), who demonstrated a mean alveolar-arterial oxygen tension gradient of 88 mm. of mercury among 10 patients with hepatic cirrhosis breathing 100% oxygen.

Four of the series of 24 patients reported by Murray *et alii* (1958) with portal cirrhosis had arterial oxygen saturation below 94%, and none of the three studied further reached normal saturation after breathing 100% oxygen at rest. We have 10 patients (Cases 1, 2, 3, 4, 5, 6, 7, 9, 11, 13) in the present series of 14 who failed to reach 98% arterial oxygen saturation breathing pure oxygen at rest, and who might at first sight be regarded as having an abnormal degree of veno-arterial shunting of blood. However, in subjects with chronic respiratory disease, there may still be a significant amount of nitrogen in some parts of the lung after breathing 100% oxygen for 10 minutes (Cournand *et alii*, 1941), and the effect of ventilation-perfusion ratio inequality will not be completely neutralized (especially if one is attempting to correct for dissolved oxygen).

The main object of exercise during the breathing of oxygen was to overcome by hyperventilation any remaining ventilation-perfusion inequality. But other possible effects which cannot be ignored might include (i) an increased arterio-venous oxygen difference, so that the effect of a given shunt on arterial oxygen saturation will be greater, and (ii) changes in the relative amount of blood shunted consequent upon circulatory adjustments to exercise. Thus the patient in Case 11 had clinical evidence of chronic respiratory disease, and functional evidence of airway obstruction and ventilation-perfusion inequality. His arterial blood oxygen saturation at rest was 92·2%. Breathing 100% oxygen raised his arterial oxygen saturation to only 96·6% (abnormally low by the standards of Wood, 1949); but exercise of moderate degree during the breathing of 100% oxygen raised it to 98·0%. The two possible interpretations are (i) that hyperventilation with oxygen on exercise overcame the effect of severe residual ventilation-perfusion inequalities, or (ii) that the relative amount of true veno-arterial shunting was significantly reduced on exercise. We offer no evidence against the former and more conservative of the two interpretations, and do not offer this case as an example of abnormal veno-arterial shunting. On the criterion of persistent and significant arterial oxygen unsaturation during exercise on pure oxygen, it is apparent that six patients (Cases 1 to 6) certainly have abnormal veno-arterial shunts, and that four patients (Cases 7 to 10) may have them. It is equally certain that four patients (Cases 11 to 14) have no gross veno-arterial shunts.

There is a limited number of sites at which the pressure gradients and anatomy are such that veno-arterial shunting of blood is possible. Excluding congenital cardiac defects, of which there was no evidence in any patient, the possibilities are limited to shunts from a high-pressure portal vein to pulmonary veins or left auricle, and direct shunting from pulmonary arteries to pulmonary veins. Channels through which shunting could occur have been demonstrated anatomically in both these sites in some patients with chronic liver disease (*vide supra*).

It is impossible by the techniques of the present study to distinguish with certainty between the two. It is probable that both varieties may contribute to arterial oxygen unsaturation in a given patient; but our results provide some indirect evidence that a major part of the shunt is intrapulmonary. One patient (Case 3) had had a large-calibre splenorenal anastomosis, found to be patent at autopsy, which should have reduced the portal vein-left auricular pressure gradient, and it is suggested that the major veno-arterial shunt in her case was probably intrapulmonary. Among the patients (Cases 1 to 6) in whom moderate shunts are certainly present, four (Cases 1, 2, 3, 5) showed a fall in arterial oxygen saturation when exercising on pure oxygen compared with resting figures on pure oxygen, one (Case 4) showed no significant change, and one (Case 6) did not exercise. In none of these patients was there a rise of saturation. A fall of saturation under such circumstances could be due to an increased arterio-venous oxygen difference with a fixed or increased percentage of cardiac output shunted, or to an increased

percentage shunt with an unchanged arterio-venous oxygen difference, or to a combination of both. It has been shown (Wade *et alii*, 1956) that exercise of the severity used in the present study leads to a decrease in splanchnic blood flow, with little or no change in splanchnic arterio-venous oxygen difference; that is, none of the conditions for a decrease in arterial oxygen saturation from portal-pulmonary vein shunting can be fulfilled. On the other hand, arterio-mixed venous oxygen differences increase on exercise, so that the fall in oxygen saturation would be produced in the presence of intra-pulmonary shunts if the percentage of cardiac output shunted rose, remained constant or, in certain circumstances, fell a little. On these grounds it is submitted that intrapulmonary shunts are likely to be present in Cases 1 to 5 at least.

Per contra, Fritts *et alii* (1959), using radio-active krypton, were recently unable to demonstrate intrapulmonary shunts in eight patients with hepatic cirrhosis and arterial oxygen unsaturation; but their technique is a new one, and it is known from anatomical studies that such shunts may occur.

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PLASMA IRON AND IRON-BINDING CAPACITY LEVELS IN HEALTH AND DISEASE : WITH AN IMPROVED METHOD FOR THE ESTIMATION OF PLASMA IRON CONCENTRATION AND TOTAL IRON-BINDING CAPACITY[1]

E. H. MORGAN[2] and GRAEME CARTER[3]

From the Department of Biochemistry, Royal Perth Hospital, and the Department of Medicine, University of Western Australia

SUMMARY

A method of estimating the plasma iron concentration and the total iron-binding capacity, which has been found suitable for routine use in a hospital laboratory, is described. It is adapted from previously described methods. The iron is released from plasma iron-binding protein by the action of dilute hydrochloric acid and thioglycollic acid, the plasma proteins are precipitated by trichloracetic acid without heating and the iron concentration is determined colorimetrically using the water-soluble reagent, sulphonated 4 : 7 diphenyl 1 : 10 phenanthroline. Total iron-binding capacity is estimated by first adding iron to the plasma in excess of the iron-binding capacity, removing the excess iron with powdered magnesium carbonate, and estimating the iron remaining in the same manner as for plasma iron.

The results obtained on the plasma from patients with infections, malignant disease, diseases of the liver and kidney, rheumatoid arthritis, hæmochromatosis, transfusional hæmosiderosis and various types of anæmia, as well as from normal persons and pregnant women, are presented. The physiological basis of the changes observed in the values measured is discussed.

It is the purpose of this paper to present a method for estimating plasma iron concentration and total iron-binding capacity and also the results obtained during a period of twelve months. The method is simple to perform, gives highly reproducible results and uses a very sensitive iron reagent. It has been found entirely satisfactory for routine use in a large hospital laboratory.

MATERIAL AND METHODS

Plasma

In this series, blood was obtained by venipuncture, which was performed on non-fasting patients always between 9 and 10 a.m. Heparin was used as an anticoagulant. The plasma was immediately separated by centrifugation. Jaundice or cloudiness of the plasma did not prejudice the assay. All glassware was washed in dilute hydrochloric acid and rinsed with glass-distilled water.

[1] Received on February 19, 1960.

[2] Resident Medical Officer, Royal Perth Hospital. Present address : Department of Physiology, University of Western Australia.

[3] Chemist, Department of Biochemistry, Royal Perth Hospital.

Principle of the Method

The principle of the method depends on the work of several authors (Trinder, 1956 ; Peters *et alii*, 1956 ; Ramsay, 1957).

Plasma Iron Concentration.—The iron is released from transferrin by the action of acid assisted by a reducing agent. After precipitation of the proteins, the iron concentration is determined colorimetrically, using the highly sensitive and water-soluble reagent sulphonated 4 : 7 diphenyl 1 : 10 phenanthroline.

Total Iron-Binding Capacity.—The plasma transferrin is first saturated by adding an excess of iron, then the iron in excess of the binding capacity is removed by adsorption to magnesium carbonate. The iron remaining firmly bound to transferrin is determined as described for plasma iron.

Reagents

Eight reagents are used, as follows :

(1) Hydrochloric acid—0·2N and 0·3N solutions.
(2) Thioglycollic acid (92%).
(3) Trichloracetic acid (30% aqueous solution, W/V).
(4) Iron reagent—sulphonated 4 : 7 diphenyl 1 : 10 phenanthroline. This is prepared as follows (Trinder, 1956) : 0·5 ml. of chlorosulphonic acid is added to 100 mg. of 4 : 7 diphenyl 1 : 10 phenanthroline (bathophenanthroline), and the mixture is boiled for 30 seconds over a Bunsen burner pilot flame. The solution is cooled and 10 ml. water are slowly added, after which

it is heated in a boiling water-bath until the precipitate completely dissolves. The volume is brought to 100 ml. with water and 1 ml. of thioglycollic acid is added.

(5) Ferric chloride—an aqueous solution containing 5 μg. of iron per millilitre in N/200 hydrochloric acid.

(6) Sulphuric acid (50% aqueous solution W/V, using sulphuric acid S.G. 1·84).

(7) Magnesium carbonate levis (powdered).

(8) Sodium acetate (40% aqueous solution W/V).

Estimation of Plasma Iron Concentration

This is performed on two-millilitre samples of plasma. Concurrently a two-millilitre sample of distilled water is treated in the same way to serve as a reagent blank.

(1) Add 3 ml. of 0·2N hydrochloric acid and one drop of thioglycollic acid to 2 ml. of plasma, mix, and allow to stand at room temperature for 30 to 60 minutes.

(2) Add 1 ml. of 30% trichloracetic acid and thoroughly mix with a stirring rod. Allow to stand for 15 to 30 minutes.

(3) Centrifuge for 15 minutes at approximately 3000 g.

(4) Pipette off 4 ml. of supernatant. To this add in turn 0·2 ml. of iron reagent, 0·6 ml. of 40% sodium acetate and 0·4 ml. of 50% sulphuric acid, mixing the solution after each addition.

(5) Read the optical density of the solutions in a spectrophotometer at a wave-length of 535 mμ or in a photometer using an " Ilford 624 " green filter.

(6) Subtract the optical density value of the reagent blank solution from the optical density value of the plasma extract solution. The resulting value is the optical density proportional to the iron content of the plasma. The iron content is obtained by reference to a calibration graph.

Individual instruments must be calibrated in the following way.

Appropriate dilutions of a standard iron solution are treated in the manner described for plasma iron concentration. A reagent blank is also prepared. Subtraction of the optical density value of the blank solution from those of the dilutions of the standard give the optical densities of solutions of known concentrations of iron. From these values a calibration graph is prepared.

Estimation of Total Iron-Binding Capacity of Plasma

This is performed on two-millilitre samples of plasma, and again a two-millilitre sample of distilled water is treated in the same way to serve as a reagent blank.

(1) Add 4 ml. of the ferric chloride solution (5 μg. of iron per millilitre) to 2 ml. of plasma, mix, and allow to stand for five minutes.

(2) Add 400 mg. of magnesium carbonate levis, and mix by frequent inversion for 30 to 60 minutes. (The magnesium carbonate can be measured by volume in a graduated test tube—100 mg. occupies approximately 1 ml.)

(3) Centrifuge for 15 minutes at 2000 g.

(4) Pipette off 3 ml. of supernatant with a Pasteur pipette, with care not to disturb the magnesium carbonate.

(5) Add 2 ml. of 0·3N hydrochloric acid and 1 drop of thioglycollic acid.

(6) Thereafter proceed as for plasma iron from Step 2 onwards.

(7) The optical density value proportional to the total iron-binding capacity of the plasma is obtained by subtracting the value of the reagent blank solution from that of the iron saturated plasma extract.

The reproducibility of replicate estimations was determined on 34 duplicate samples of plasma for both plasma iron concentration and total iron-binding capacity. A coefficient of variation of 3·70 for plasma iron concentration and 2·59 for total iron-binding capacity determinations was found.

Results

The results of plasma iron concentration and total iron-binding capacity estimations performed on blood from normal persons,

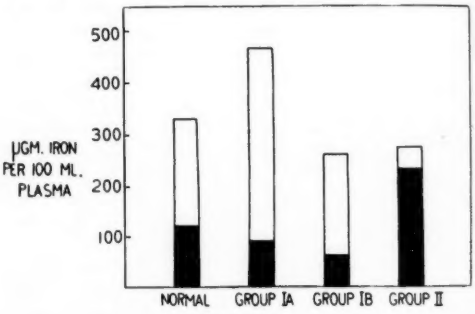

FIGURE I

Plasma iron concentration and iron-binding capacity in normal subjects and in a variety of diseases arranged as described in the text and shown in Table I. The total height of the columns indicates the mean total iron-binding capacity, the height of the shaded portion of the columns the mean plasma iron,

pregnant women and patients with a variety of clinical disorders are presented in Table I. The patients' results were divided into two main groups. Patients in Group I had plasma iron values below normal, and those in Group II had values above normal. Group I was further subdivided into Group IA, with increased total iron-binding capacity values and Group IB, with decreased values. In Figure I the mean plasma iron concentration and iron-binding capacity values of the different groups are shown diagrammatically.

Discussion

The methods given in this paper for plasma iron concentration and total iron-binding capacity embody some of the more satisfactory features of the methods described by Trinder (1956), Peters *et alii* (1956) and Ramsay (1957).

TABLE I

Plasma Iron Concentration and Total Iron-Binding Capacity in Normal Subjects, Pregnancy and Various Diseases

Group	Diagnosis	Number of Subjects	Plasma Iron Concentration (Microgrammes per 100 ml.)		Total Iron-Binding Capacity (Microgrammes per 100 ml.)	
			Mean	Range	Mean	Range
Controls:						
Male ..	No abnormality	35	127	67–191	333	253–416
Female ..	No abnormality	35	113	63–202	329	250–416
Group IA[1]	Pregnancy:					
	First trimester	27	123	53–172	370	242–478
	Second trimester	115	98	24–180	428	294–674
	Third trimester	106	94	22–185	532	373–712
	Iron deficiency	35	32	0–78	482	304–705
Group IB[2]	Infections	11	47	30– 72	260	182–370
	Carcinoma	6	42	22– 60	241	144–300
	Renal insufficiency	5	75	40–125	239	200–286
	Cirrhosis of liver	8	86	56–132	281	182–390
	Rheumatoid arthritis	3	59	46– 80	226	212–246
	Treated megaloblastic anæmia	10	46	24– 65	288	195–390
Group II[3] ..	Hæmochromatosis	14	250	191–290	263	205–330
	Transfusion hæmosiderosis	5	230	178–257	272	220–312
	Infective hepatitis	4	230	191–295	349	226–420
	Untreated megaloblastic anæmia	4	197	158–235	231	226–235
	Aplastic anæmia	4	226	185–272	272	238–308
	Hæmolytic anæmia	4	190	148–227	258	196–352

[1] Low plasma iron concentration, high total iron-binding capacity.
[2] Low plasma iron concentration, low total iron-binding capacity.
[3] High plasma iron concentration

Trinder (1956) showed that sulphonation of bathophenanthroline produces a stable water-soluble compound. This forms a red-coloured iron complex with Fe^{++} of specific extinction two to three times as great as that of other iron reagents, and thereby increases sensitivity. The addition of the sodium acetate adjusts the pH so that complete and stable colour formation occurs. Only copper of the other metals in blood gives a colour with it, and this is destroyed by the sulphuric acid. Peters *et alii* (1956) found that the addition of hydrochloric and thioglycollic acid, allowing the solution to stand for 30 minutes and then adding the trichloracetic acid, as described above, resulted in the production, without heating, of an optically clear solution, with a mean recovery of added iron of 101·5% (standard deviation 2·8%). Ramsay (1957) found that magnesium carbonate levis added to plasma did not remove iron in significant amounts from transferrin, but did remove iron present in excess of the total iron-binding capacity. This was true under the conditions used in the method described here for amounts of iron up to 1600 μg. per 100 ml. of plasma.

The plasma iron concentration is decreased in a considerable variety of diseases and in pregnancy. In all these conditions anæmia occurs commonly, but it is only in iron-deficiency anæmia and probably in pregnancy that the anæmia is due to lack of iron, and only here that iron therapy is beneficial. In these two states the total iron-binding capacity is elevated, while in other states associated with low plasma iron concentration the total iron-binding capacity is sometimes normal, but in most cases low. Hence determination of total iron-binding capacity in conjunction with plasma iron concentration is essential in evaluating the significance of a lowered plasma iron concentration and as an aid in the diagnosis of anæmias which cannot be differentiated by standard hæmatological methods.

It is probable that the changes which occur in pregnancy, being similar to those in iron-deficiency anæmia, are in part due to iron deficiency, because of the great demands made on the mother's iron store by the fœtus and by the increase in her own circulating hæmoglobin mass. That this is not the whole explanation with regard to iron-binding capacity is suggested by the finding of Gerritsen and Walker (1954) that among the Bantu, whose iron reserves are greatly increased owing to dietary causes, pregnant women do not show a fall in plasma iron concentration, but do show a rise in iron-binding capacity. This is not of the same magnitude as found in the present material, but is nevertheless quite marked. The mechanism of the rise in iron-binding capacity found in iron deficiency is unknown.

The changes in iron metabolism which occur in infections have been extensively studied by Cartwright and his colleagues, and their results have been summarized by Cartwright and Wintrobe (1955). They consistently observed low levels of plasma iron

and iron-binding capacity, a decreased rate of utilization of iron for hæmoglobin synthesis and a rapid rate of removal of intravenously injected iron from the blood-stream. A reduction in plasma iron concentration occurs early in the course of an infection. The mechanisms of these alterations in iron metabolism are unknown. There is considerable evidence that the decrease in plasma iron concentration is not due to the reduction in iron-binding capacity (Cartwright and Wintrobe, 1955). Abnormally rapid removal of iron from the plasma by the reticulo-endothelial system in infections has been considered. This is unproven. Intravenously injected radioactive iron is removed mainly by the liver (Greenberg *et alii*, 1947), but it is not known to what extent the liver parenchymal or reticulo-endothelial cells are involved.

In malignant conditions, the plasma iron concentration is usually low whether anæmia is present or not (Laurell, 1947). Progress of the disease is accompanied by a falling plasma iron concentration (Miller *et alii*, 1956) and these workers observed a rise in plasma iron concentration in two patients with therapeutic remissions. In one patient examined by the present writers, the plasma iron level was 122 μg. per 100 ml. one week before laryngectomy for carcinoma of the larynx. Four weeks after the operation it was 274 μg. per 100 ml., and the total iron-binding capacity 281 μg. per 100 ml. In the meantime the diagnosis of hæmochromatosis had been made and confirmed by liver biopsy. It is possible that serial plasma iron estimations may have some place in assessing the effectiveness of treatment of malignant conditions.

As in infections and malignant disease, the mechanism of the changes in plasma iron and iron-binding capacity in rheumatoid arthritis, cirrhosis and renal failure are unknown. In all these conditions there occur profound metabolic alterations, including changes in protein metabolism, anorexia and a resultant poor intake of essential foodstuffs, and commonly a reduction in the rate of erythropoiesis. These factors probably play some part in the changes observed in iron metabolism.

Transferrin is a protein of relatively low molecular weight (90,000). In the presence of considerable proteinuria it is readily lost, with its bound iron, in the urine, and this loss may play some part in the development of the very low plasma iron and iron-binding capacity values found in the nephrotic syndrome (Cartwright *et alii*, 1954). In one case of Type II nephritis studied in this laboratory, the plasma iron concentration was 80 μg. per 100 ml., and the total iron-binding capacity 185 μg. per 100 ml.

Occasionally low plasma iron and iron-binding capacity levels may be observed when no abnormality can be found in the patient apart from malnutrition. One such patient with anorexia nervosa had a plasma iron concentration of 39 μg. per 100 ml. and a total iron-binding capacity of 204 μg. per 100 ml. In experimental animals with protein deficiency, a decrease in both values has been found (Cartwright and Wintrobe, 1948).

Elevation of the plasma iron concentration may occur in several different diseases. In all of these except hepatitis, the total iron-binding capacity is usually moderately low. In hepatitis, the total iron-binding capacity may be low, normal or elevated. In hæmochromatosis and transfusion hæmosiderosis, the elevation of plasma iron level is due to the greatly increased iron stores. In hæmochromatosis treated by venesection, the plasma iron level falls to normal when the iron stores reach approximately normal levels. Determination of the plasma iron concentration can be of value in assessing the necessary frequency of bleeding.

The elevated plasma iron concentration in untreated megaloblastic anæmia and in aplastic anæmia is thought to be due to the bone marrow's inability to utilize the iron normally. In hæmolytic anæmia, the elevation is due to increased release of iron from hæmoglobin as a result of decreased erythrocyte survival time. Within 48 hours of the commencement of specific treatment in the megaloblasic anæmias, the serum iron falls to a low level, owing to the greatly increased erythropoiesis utilizing the iron very rapidly (Hawkins, 1955). Similarly a fall occurs in hæmolytic anæmia during recovery from a hæmolytic crisis.

In experimental hepatocellular necrosis, a rise in plasma iron concentration, of degree proportional to the amount of cellular damage, has been observed (Reissmann *et alii*, 1954). This rise is thought to be due to release of storage iron from the damaged liver cells. A similar mechanism is probably responsible for the elevation of plasma iron level in infective hepatitis, and estimation of the plasma iron level can be of some value in following the course of the hepatocellular damage. In obstructive jaundice, no rise in plasma iron level is observed. Because of this, plasma iron estimation has been advocated by several authors as a useful

guide in the differentiation of hepatocellular and obstructive jaundice.

An apparently greatly elevated plasma iron concentration may be observed for some days after parenteral iron therapy. This is due to the presence in the blood of iron in a colloidal form not bound to transferrin. In six such cases, plasma iron determinations performed within a week of intramuscular injections of iron dextran gave values ranging from 540 to 925 μg. per 100 ml. It is apparent that estimation of plasma iron concentration is of little value for approximately two weeks after intramuscular iron therapy.

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"Q" FEVER : CLINICAL FEATURES IN 72 CASES[1]

OWEN POWELL[2]
From the Princess Alexandra Hospital, Brisbane

SUMMARY

In 72 consecutive cases of "Q" fever, with no death, the commonest symptoms were headache and anorexia. Myalgia, sore eyes, cough, chills and sweats were also frequent. The commonest signs were a relative bradycardia, hepatomegaly and splenomegaly. Minor chest signs were frequently found. The total duration of fever was 10 days or less in the majority of cases. The longest duration of continuous fever was 24 days, and of intermittent fever 34 days.

Common laboratory findings included an increased erythrocyte sedimentation rate, the presence of atypical mononuclear cells during the acute illness and an absolute lymphocytosis during convalescence. Only three patients had radiologically demonstrable pneumonic changes. However, respiratory infection of a lower severity was frequent, and this suggests that local strains of *Coxiella burneti* may be less virulent than overseas strains. Subclinical involvement of the liver was common, and in seven cases there was electrocardiographic evidence of transient myocardopathy.

About 70% of patients returned to work within six weeks from the onset, but the convalescence of the remainder was protracted. The duration of convalescence was prolonged with increasing age. Infection with *C. burneti* may become chronic.

Cases are described in which "Q" fever was complicated by pneumonia, pleural effusion, thrombocytopenia, encephalitis and endocarditis, and in which the problem of chronicity is illustrated.

SINCE the original description by Derrick (1937) of a febrile illness affecting abattoir workers and dairy farmers in south-eastern Queensland, an extensive literature has grown on the subject of "Q" fever. For the most part, and particularly on the clinical aspects, this has been from overseas sources. After the recognition of the disease in the United States, and later amongst troops in the Mediterranean area, especial emphasis was placed upon the pulmonary features (Hornibrook and Nelson, 1940; Finland and Dingle, 1942; Robbins and Ragan, 1946). Subsequently it has become appreciated that "Q" fever is a generalized disorder which may involve the lungs, the pericardium (Ludwig, 1956), the central nervous system (Clark *et alii*, 1951) and the liver (Douglas, 1956; Tonge and Derrick, 1959). Furthermore, although this is insufficiently realized, it may progress to a chronic form (Marmion *et alii*, 1953), and in some chronic cases the heart valves may be involved (Evans *et alii*, 1959; Robson and Shimmin, 1959; Andrews and Marmion, 1959).

"Q" fever has increased in incidence in Queensland over the past three years. There were 76 cases notified in 1956–1957, 194 in 1957–1958, and 420 in 1958–1959. This increase was in part due to involvement of the sheep industry, but particularly to the recent development of the packaged beef trade with the United States. Nevertheless, there appears to have been also an increased incidence in the known endemic areas (Tonge, 1960). For many years the State Laboratory of Microbiology and Pathology has provided practitioners in Queensland with a service whereby a battery of serological investigations is carried out on blood from patients with undiagnosed fevers. The medical profession in this area has been well aware of the existence of "Q" fever for many years. Moreover, it is a compensatable disease, and workmen in exposed industries are, naturally, alert to the possibility, and do not hesitate to raise the question. It seems probable, therefore, that the proportion of cases being diagnosed is not markedly greater than in previous years. In other words, the increase is real, and not merely due to increased diagnosis. It therefore seems appropriate to review the clinical features of the disease as it appears locally.

MATERIAL

This is a consecutive series of 72 cases of "Q" fever diagnosed in the public wards of this hospital over a period extending from late July, 1958, until early June, 1959, but excluding two periods totalling five weeks in December and April.

[1] Received on February 25, 1960.
[2] Medical Superintendent.

All patients were male. Of the total, 64 were abattoir-workers, two worked in shearing sheds, five were from a wool-scour, and one was a dairy-farmer. The age distribution was as shown in Table I.

Diagnosis in all cases was confirmed by the demonstration of complement-fixing antibodies to *C. burneti*. Either a rise in titre from zero

TABLE I

Age (Years)	Number of Cases
0–19	22
20–29	15
30–39	14
40–49	9
50–59	9
60–69	2
70 and over	1
Total	72

to 1 in 32 or greater, or a titre in a single specimen of 1 in 256 or greater in patients admitted to hospital or suspected of this infection late in the illness, was considered diagnostic.

The following investigations was performed on all patients, with a few exceptions: X-ray examination of the chest, estimation of the erythrocyte sedimentation rate, a differential leucocyte count, microscopic examination of the urine, electrocardiography, and a variety of liver-function tests. The results of the last-mentioned and a survey of liver involvement will be given separately.

Onset

The onset was acute in almost every case, and the patient could remember the commencement of the illness clearly, usually to within a few hours. An occasional one could recall the precise time when symptoms first occurred. The interval between onset and taking to bed varied from a few hours to two days, a few men having continued to work for several days. It was a common observation that in the first 48 hours symptoms abated in the morning, only to return within a few hours; but in practically all cases symptoms were persistent after this period. Three patients noted prodromal symptoms of vague malaise and occasional headache for several days before the acute onset. The time elapsing from onset to hospital admission is shown in Figure I. It will be seen that patients were usually admitted during the first week, and most commonly on the fourth day of the illness. One's impression was that hospital admission was sought because of continuation rather than aggravation of the symptoms. To this extent it is possible that mild cases could pass undiagnosed because the patient failed to report.

Symptoms

The initial symptoms were usually chilly sensations and malaise, accompanied or closely followed by headache. Figure II illustrates the frequency of the various symptoms complained of in this series. Headache, most often frontal, was usually quite severe and the major complaint. However, in only a few cases was it of the incapacitating severity commonly described in the literature. It was only poorly relieved by aspirin. Anorexia was the next most common symptom, and was of the degree which one observes in viral hepatitis. It was associated with a distaste for tobacco, although one patient noted this symptom in the absence of anorexia. Nausea, vomiting and abdominal pain were relatively common, but rarely severe. The

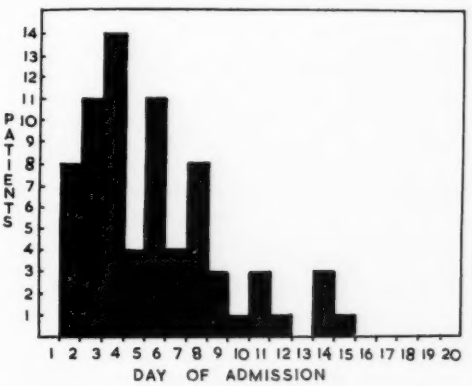

FIGURE I

Time elapsing from onset to admission to hospital. The day on which symptoms commenced is taken as Day 1, regardless of the time of day at which this occurred

pain varied from mild epigastric or mid-abdominal aching to definite, though not severe, colic. However, one patient was initially admitted to a surgical ward with a presumptive diagnosis of acute obstructive cholecystitis. Diarrhœa was severe in only one of the series. Occasional blurring of vision occurred, which in three cases persisted into convalescence. All these patients were aged over 40 years, and it appeared that the debilitating illness had brought a preexisting presbyopia to attention.

Findings on Physical Examination

Apart from fever itself, the most constant sign was a pulse rate which was slower than

would have been expected from the degree of fever. With a temperature of 103° F., for example, the pulse rate was usually about 100 per minute, or less. This relative bradycardia was an important feature, and was observed in 69 of the series (96%).

Hepatomegaly was demonstrated in 47 patients (65%), and in an additional eight there was tenderness on palpation beneath the right costal margin, although no enlargement of the liver could be detected. The enlargement was never great, its extent rarely being more than 8 cm. beyond the rib margin in the nipple line. Three patients developed jaundice in the course of the illness. Splenomegaly was present in 38 cases (53%).

Abnormal physical signs in the chest were found in 28 cases (39%). In most, these signs were minor, consisting of more or less generalized rhonchi or small patches of basal crepitations. However, in one there was evidence of a considerable pleural effusion, and three more patients had definite evidence of consolidation.

Conjunctival injection was noted in seven cases, and slight generalized lymph-gland enlargement in eight, but one of these patients had a coexisting lymphosarcoma. Slight faucial injection was seen in three cases. A rash occurred in four instances. In one it was frankly urticarial, and was probably due to drug allergy; one patient presented with purpura; and the remaining two patients showed transient maculo-erythematous eruptions of doubtful significance. In one case the tongue was red and denuded during the acute illness, returning to normal in convalescence, and this man also suffered from herpes labialis.

Neck stiffness was noted in eight cases (11%), being moderately severe in six and marked in two. The cerebro-spinal fluid was examined in several of these, but no abnormalities were found. A confusional state occurred in two patients.

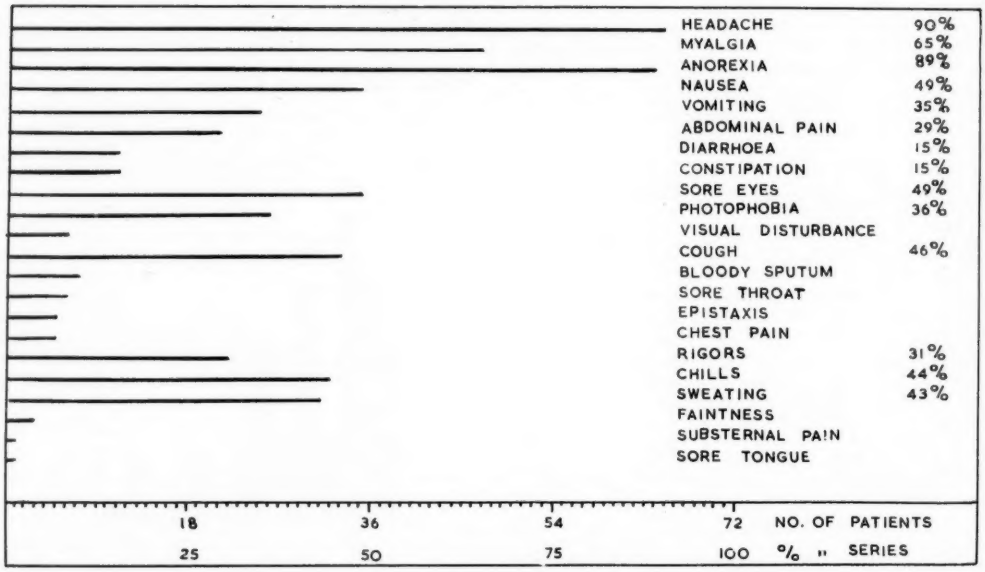

FIGURE II
Frequency of symptoms complained of in the series

FEVER

Fever was present in all cases except for one patient admitted to hospital because of purpura two days after cessation of fever. The peak temperature varied between 102° F. and 104° F., being only occasionally above this. As a rule there were quite marked daily variations, but this was not always so, and it may be that salicylates were responsible, at least in part. In the early part of the febrile period, the temperature did not as a rule fall to normal; but in a number of cases it was noticeable that the fluctuations were marked in the last two or three days of fever, the temperature returning to normal between peaks, with successive peaks lower than the one before. After this the temperature remained below normal for about

two days, and then returned to normal levels. Twelve patients experienced a secondary febrile period, as described by Derrick, and one had a tertiary fever. It is, of course, possible that some of the longer periods of continuous fever were due to a superimposed secondary fever. The duration of fever over the series is shown in Figure III. It can be seen that 70% of the series had a total duration of fever of 10 days or less. In these figures it is assumed that all patients were febrile from the day on which symptoms commenced, which is taken as Day 1. The patient with the longest continuous fever had a complicating staphylococcal infection, which was probably contributory.

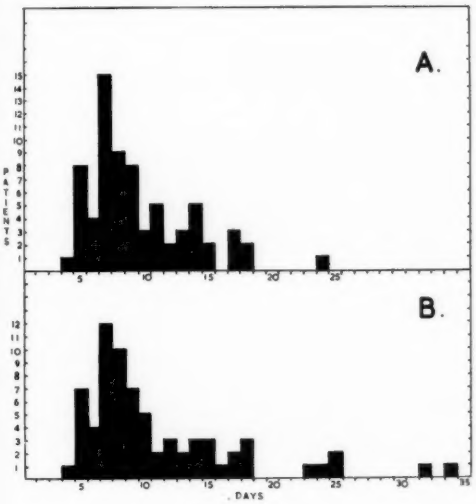

FIGURE III

Duration of continuous fever (A), and total period over which fever occurred (B). The latter thus includes the periods of "secondary" or "tertiary" fever. It is assumed that fever commenced on the day when symptoms appeared

COURSE OF ILLNESS AND CONVALESCENCE

Acute symptoms did not outlast the fever, and many patients felt comparatively well for a day or two before it abated, although return of appetite tended to be delayed. As a rule they were well enough for discharge from hospital within three or four days of the temperature returning to normal. Nevertheless, almost all complained of undue fatigue and weakness for the following one or two weeks. Occasionally there was some return of headache. Most patients were by then well enough to return to work. However, in a proportion of cases convalescence was prolonged. Such patients complained of undue fatigue, "knocking up" on moderate exertion, poor appetite and occasional headache. Accurate weight records unfortunately were not kept, but considerable loss of weight, often up to a stone, was commonplace during the acute illness, and in those whose convalescence was prolonged this was only slowly regained. Figure IV shows the period

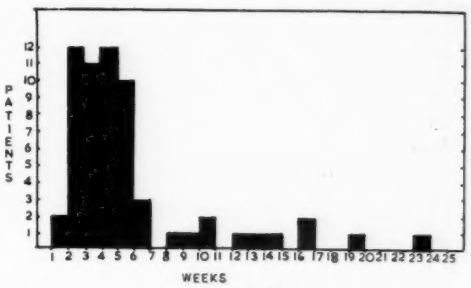

FIGURE IV

Period elapsing from onset until return to work in the 60 cases for which such records are available

from onset until return to work in the 61 cases for which this information is available. It can be seen that in 15 of the 61 (25%) the period was greater than six weeks, and in 12 (20%) it was greater than eight weeks. It is worth recording that the total amount of time on workers' compensation payments in this group amounted to 2013 days.

An analysis in regard to age is of interest. The mean period off work for the respective age groups is as follows: 0 to 29 years (31 patients), 29 days; 30 to 49 years (20 patients), 45 days; 50 to 69 years (10 patients), 68 days. This confirms previous observations that convalescence is more protracted in the elderly.

INVESTIGATIONS

Erythrocyte Sedimentation Rate

During the period in hospital, the maximum erythrocyte sedimentation rates (Cutler) recorded were as shown in Table II. As a rule, this increased rate reverted to normal in early convalescence. A persistently elevated sedimentation rate (greater than 15 millimetres in one hour) beyond the seventh week occurred in eight of the series. Four of these were in the group of 12 mentioned above with a protracted convalescence. The longest period over which the rate remained elevated was 165 days in a case reported below (Case 7), in which chronic infection seems likely.

Leucocyte Count

Figure V sets out the range of total leucocyte counts in the series, with absolute figures for

neutrophils and lymphocytes. It can be seen that, whilst there was an occasional tendency to neutropenia in the initial count, the values were generally within normal limits. However, during convalescence there was a definite lymphocytosis. In his original paper, Derrick remarked on a relative lymphocytosis in some of his patients at this stage, but subsequent reports had failed to confirm it. The lymphocytosis, which is absolute as well as relative, was usually apparent in the third or fourth week of the illness. Occasionally it appeared earlier than this, and in a few cases later. The differential counts usually returned to normal by the seventh week.

TABLE II

Erythrocyte Sedimentation Rate (Millimetres in one Hour)	Number of Cases
0–8 (normal) ..	18
9–15	22
16–20	12
21 and over	15
No record	5
Total	72

In 37 cases (51%), atypical mononuclear cells were found to make up more than 5% of the total leucocyte count on at least one occasion. The highest value recorded was 28% of a total leucocyte count of 4400. These cells were usually found during the period of fever and for a week or two afterwards.

Radiological Appearances in the Chest

The radiological picture was abnormal in only four of the series. In two there were opacities in one or other lower zones; in another there was more extensive consolidation in the lower lobe of the right lung, with very slow, but ultimately complete, clearing over many weeks; and in the fourth there was evidence of a large pleural effusion.

Electrocardiographic Findings

Electrocardiography was carried out in 50 cases on one or more occasions. Abnormal tracings were found in 10 of these. In one there were frequent ectopic ventricular contractions. Over the ensuing twelve months this patient developed frank ischæmic changes, culminating in a myocardial infarct and eventual death from cardiac failure. The ætiology of the original abnormality was presumably atherosclerosis. Premature atrial contractions occurred in another. Sinus bradycardia was seen in one (with a rate of 46 per minute), and this patient had abnormal *T* waves. Significant *T*-wave abnormalities occurred in seven cases, and consisted of flat, diphasic, or inverted waves in various leads. No *QRS* changes were seen, nor did there appear to be any interference with conduction. Figure VI illustrates typical serial tracings in one case. The changes are

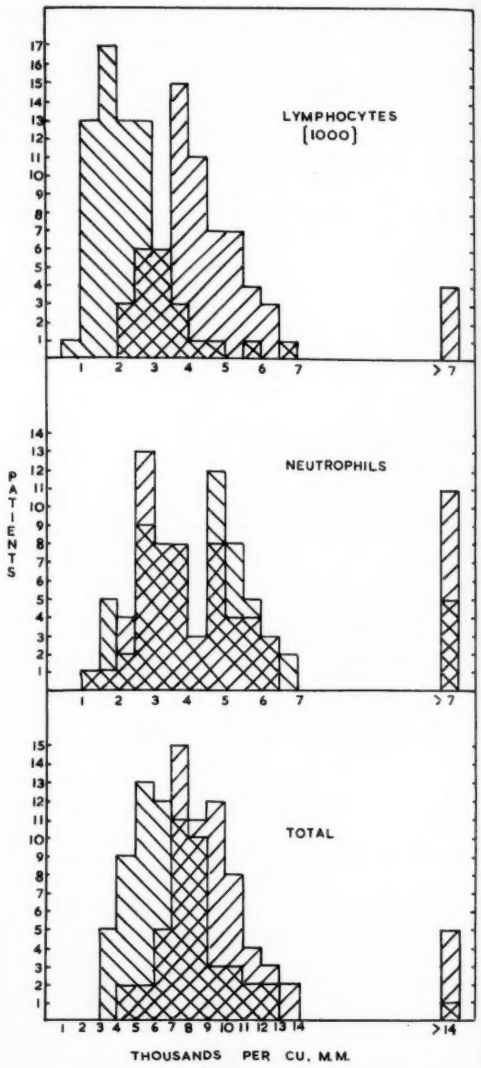

FIGURE V

Range of total leucocyte counts and absolute values for lymphocytes and neutrophils. Columns hatched left to right downwards, on admission to hospital; hatched right to left downwards, in convalescence

similar to those described in scrub typhus by Levine (1945), and are presumably evidence of transient myocardial involvement.

Microscopic Findings in the Urine

In seven of the series, microscopic examination of the centrifuged urinary deposit revealed significant numbers of erythrocytes. In five this abnormality was slight, the number varying from 10 to 15 erythrocytes per high-power field, and could well be accounted for by the fever alone; but in two cases counts of several hundred were found. In one of these the hæmaturia was a manifestation of thrombocytopenia, but in the other no alternative cause was found, and it disappeared after about 10 days. Occasional granular and hyaline casts were found in some specimens, but never in any number.

Liver Function Tests

Positive results to one or more of a variety of liver function tests were found in 56 patients (78%). These will be reported in detail separately.

Special Features

To what extent one can speak of complications in "Q" fever is debatable. It seems very likely that any organ or system may be affected by the disease to some degree, although in most cases this is not demonstrable clinically. It is proposed, therefore, to point out certain features which seem to indicate involvement of particular systems, or which highlight other important aspects of the condition.

Respiratory System

Cough, increased sputum (blood-stained in 10% of cases), generalized rhonchi and absence of radiological changes suggest that the commonest manifestation of respiratory involvement in this area is acute bronchitis. As was previously stated, only three patients of the series had radiographic evidence of pneumonia. In only one case was pneumonia a major feature of the disease (Case 72). Another patient developed a large pleural effusion.

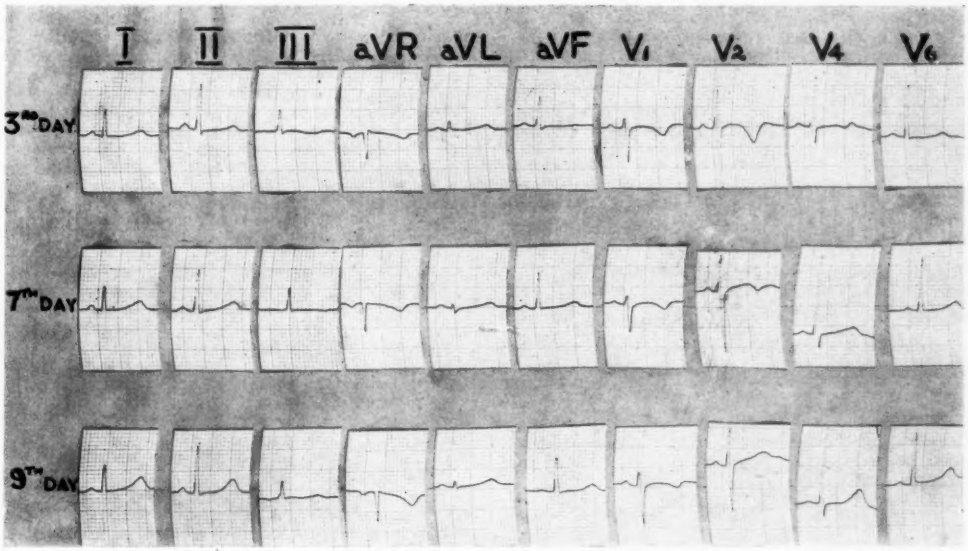

Figure VI
Serial electrocardiograms of P.G., aged 15 years (Case 46)

Hæmatopoietic System

Only one instance of possible clinical involvement of the hæmatopoietic system occurred; this patient was the man admitted to hospital with thrombocytopenic purpura (Case 30).

Central Nervous System

Although neck stiffness was not rare, abnormalities of cerebro-spinal fluid were not found. Two patients of the series developed

a confusional state. One was a man who was already moderately ill with clinical jaundice and pneumonia. The other patient had abnormal physical signs, and a true encephalitis may be inferred (Case 28).

Uro-Genital System

Apart from the microscopically evident hæmaturia mentioned earlier, and the occasional presence of albuminuria, which was probably febrile in origin, no other evidence of renal involvement was obtained.

Gastro-Intestinal System

One man was admitted to hospital with profuse diarrhœa, and, in fact, was initially sent to an isolation ward with the diagnosis of a possible Salmonella infection. It had been present for one week before his admission, and persisted for a further four days.

The Liver

Three patients in the series became jaundiced. Other evidence suggested that sub-clinical liver involvement was extremely common.

Cardio-Vascular System

Apart from electrocardiographic abnormalities, there was no other evidence of cardio-vascular involvement in the series. However, one patient with probable endocarditis, who was in hospital before this investigation was commenced, is included because of the importance of this aspect (patient W.McC.).

Chronicity

Persistence of an elevated erythrocyte sedimentation rate suggests the possibility of continuing infection, particula ly in patients with a protracted convalescence. In only one such case could confirmatory evidence be obtained. In this case, urine obtained on the hundred and seventh day was inoculated into guinea-pigs, which subsequently developed complement-fixing antibodies to *C. burneti*. In yet another, in which the serum alkaline phosphatase level was persistently raised, liver biopsy on the hundred and twenty-third day demonstrated the presence of an inflammatory focus in the liver. Two other cases, which are not included in the series, are briefly reported below, as these provide further evidence of chronic infection (patients W.McC. and J.W.).

REPORTS OF CASES

CASE 72.—B.G. was admitted to hospital on the eighth day of an illness which had commenced with headache, nausea, abdominal cramps and fever. Subsequently, cough with blood-streaked sputum had developed. He had received a variety of antibiotics before his admission. When he was first examined, there was clinical evidence of right lower lobe pneumonia, and this was confirmed radiographically. Subsequently his spleen became palpable. A leucocyte count three days after his admission to hospital revealed 8600 cells per cubic millimetre, of which 78% were neutrophils. There were numerous leucocytes and Gram-positive diplococci in the sputum. Regarded at first as suffering from pneumococcal pneumonia, he was treated with penicillin in a dosage of 500,000 units every six hours, with no effect on his fever. Subsequently streptomycin was added in a dose of one gramme *per diem*, again without noticeable effect, although his fever finally abated on the eighth day in hospital. Blood taken on the sixteenth day of the illness revealed complement-fixing antibodies at a titre of 1 in 32, and in a specimen on the twenty-third day the titre was 1 in 256. When he was discharged from hospital, there had been very little clearing of the lesion, as shown by radiographic examination of the chest. At subsequent follow-up examination in the out-patient department, little evidence of resolution was observed, and a bronchoscopic examination was carried out by Dr. C. Lomas. No abnormality of the bronchial tree was seen, and examination of the sputum revealed no malignant cells. Finally, complete clearing occurred approximately four months after the original onset.

CASE 12.—J.McI. was admitted to hospital on the ninth day of a typical illness. There had been some cough for the preceding twenty-four hours, with "black" sputum. On examination of the patient, there were generalized rhonchi, with a few coarse crepitations at the base of each lung, and a radiographic examination of the chest revealed no abnormality. His fever abated on the eleventh day, and he was discharged from hospital on the thirteenth day. On the fifteenth day he had a recurrence of his original symptoms of headache, fever and rigors, with a slight cough, and aching pain in the left upper quadrant of his chest. On the seventeenth day he was readmitted to hospital, these symptoms having persisted. On that day he had noticed some blood in his sputum. No abnormal clinical signs were detected on examination of his chest, and another X-ray film of the chest showed normal appearances. There was no evidence of peripheral venous thrombosis. On the twenty-first day it was apparent that he was developing a left-sided pleural effusion, which became quite large in the course of two days. Aspiration produced turbid, dark amber fluid containing numerous erythrocytes and moderate numbers of leucocytes, of which 60% were mononuclear cells and 40% polymorphs. The effusion required one further aspiration, and had completely resolved by the thirty-eighth day.

In neither of these cases can "Q" fever be blamed with certainty for the pulmonary features. In the first, the patient had undoubted "Q" fever, but there is a reasonable possibility that the pneumonia was a complicating pneumococcal infection, and the delayed resolution may support this theory. In the second, it cannot be denied that a pulmonary infarct may have followed a disease which may well predispose to thrombus formation. Nevertheless, the definite recrudescence of the original symptoms, and the absence of radiographic evidence of an infarct three days after the incident, do offer some basis for regarding this

as a primary "Q" fever pleural effusion. However, inoculation of pleural fluid into guinea-pigs failed to verify the presence of the organism.

CASE 30.—R.McI. was admitted to hospital eight days after the onset of fever, which was accompanied by malaise, generalized myalgia, neck stiffness and drowsiness. He had been treated before his admission with one injection of 1,000,000 units of procaine penicillin, followed by an oral course of penicillin for several days. He had also taken aspirin intermittently. On the day of his admission to hospital he had developed a purpuric rash over the face, tongue, trunk and limbs, and had passed a melæna stool. In addition, the spleen was palpable, and there was a strongly positive response to the Hess test. Investigations gave the following results. The hæmoglobin value was 13·0 grammes per 100 ml.; the leucocytes numbered 11,300 per cubic millimetre (neutrophils 46%, lymphocytes 52%, monocytes 1%, eosinophils 1%, and a few atypical mononuclear cells); no platelets were seen in the smear, and a direct platelet count the following day again revealed no platelets. The response to the Paul-Bunnell test was negative. Three days after the patient's admission to hospital, his hæmoglobin value had fallen to 9·0 grammes per 100 ml., and a direct transfusion of 500 millilitres of blood was given. Prednisone therapy was commenced on the day after his admission to hospital, with a dose of 60 mg. *per diem*. On the fourth day after his admission, one giant platelet was found in the peripheral blood, and one was found the following day. On the sixth day, the platelet count was 18,000 per cubic millimetre, and thereafter it rose daily to a level of 200,000 per cubic millimetre on the tenth day after his admission. Prednisone therapy was stopped on the eighteenth day, after gradual withdrawal, and the patient remained well subsequently. The result of the complement-fixation test was negative on his admission, but a titre of 1 in 256 was obtained during early convalescence.

This patient had undoubted "Q" fever. There is no way of deciding whether the thrombocytopenia was due to this, or to the therapy prior to his admission to hospital, or was a latent idiopathic thrombocytopenia. It has not before, to my knowledge, been reported in "Q" fever.

CASE 28.—R.Kn. was an epileptic of many years' standing, who had had no seizures in the year before his admission to hospital, which was on the fourth day of a typical illness. Fever, with a temperature reaching 105° F. on one occasion, lasted until the tenth day of the illness, and he was discharged, well, on the fourteenth day. Owing to oversight, he did not receive his usual anti-convulsant drugs when in hospital, but it is believed that he recommenced taking them on his return home. Three days after his discharge, he was readmitted to hospital with the history that he had had a convulsive seizure that morning, and had become unconscious. He was feverish (temperature 101 °F. on his admission to hospital), but it was not known when the fever had returned. On examination, he was apparently aware of his surroundings, but would neither speak nor answer questions. He was having frequent focal fits involving the right side of the face, combined with deviation of the head and eyes to the right. There was an extreme degree of neck stiffness, but it was very difficult to say how much of this was due to voluntary resistance. The fundi were normal, and at that time there were no other neurological signs. Lumbar puncture produced normal cerebro-spinal fluid under normal pressure. His condition deteriorated over the next 48 hours. He was having very frequent fits of the type described, and although he lay with his eyes open, apparently conscious, it was obvious that he was quite unaware of his environment. A left carotid angiogram was performed, and revealed no abnormality. He had been receiving "Dilantin" (90 mg. four times a day) since his admission to hospital. By the fourth day of his readmission, it was believed that the fits were less severe, and improvement continued. By the sixth day, the fever was abating, and he would obey instructions, but was unable to speak. Two days later he was very much better. At this time definite localizing signs were found—namely, increased tone and deep tendon reflexes with an equivocal plantar response on the right side, and he had a moderate degree of expressional aphasia. These signs persisted for a further week. After his discharge from hospital he required prolonged convalescence. During this time he complained of vagueness, inability to concentrate and impairment of memory, to the extent that at midday he was unable to remember the details of his breakfast. Slow improvement took place, until after three months he had in all respects returned to normal.

Despite the history of epilepsy, it is thought that the duration of abnormal physical signs after recovery, and in particular the persistent intellectual defect, indicate that this was in fact a true encephalitis. It is regretted that electroencephalography could not be carried out.

CASE 7.—W.H., during his period in hospital, had biochemical evidence of liver involvement, and this was confirmed by needle biopsy. After his discharge he remained vaguely unwell for a considerable time, although no definite physical abnormality could be found. Apart from persistently positive results to flocculation tests, which observation is not unusual, the only other feature was a continued elevation of the erythrocyte sedimentation rate. Urine from this man, obtained towards the end of the third month, on inoculation into guinea-pigs caused the production of complement-fixing antibodies to *C. burneti*. This test was repeated later, but on this occasion the result was negative. His erythrocyte sedimentation rate 165 days after the onset was 19 mm. in one hour. At that stage he was lost sight of, although he was still not well. He was seen again 41 weeks after the onset, when he had apparently completely regained normal health.

The two following cases are not included in this series.

W.McC. was admitted to hospital in May, 1957, with a history of intermittent fever for the preceding twelve months. His feet had been swelling for some months, and he was receiving digoxin and mersalyl. He stated that he had had "Q" fever in 1945. On examination, he had clinical evidence of severe aortic incompetence, a large tender liver, a palpable spleen, and clubbing of the fingers. There was no evidence of peripheral emboli. The leucocytes numbered 7500 per cubic millimetre, of which 75% were neutrophils. Repeated blood cultures failed to yield any pathogens. His condition deteriorated, and he died three months

after his admission to hospital, in spite of treatment with various antibiotics. Complement-fixation tests to *C. burneti* were performed on two separate occasions, and antibodies were present in a titre of 1 in 512 or greater in both specimens of serum. Autopsy was not performed until four days after death, and revealed an aortic valve which appeared to have been the site of old rheumatic disease, but which had suffered considerable destruction from endocarditis. Friable vegetations were present. *Staphylococcus aureus* was grown from the aortic valve, although no bacteria were seen in sections of the valves, and it is quite possible that this was merely a result of the delay between death and autopsy. Eighteen months later, the sections were reviewed by the Laboratory of Microbiology and Pathology, which reported that organisms were present which resembled *C. burneti*.

J.W. was admitted to hospital in March, 1959, with a history typical of "Q" fever. Exactly six years previously he had had a proven attack of "Q" fever, complement-fixing antibody titres on that occasion having risen from zero to 1 in 128. On very close questioning, he admitted that since that time he had been a little less well than previously, but it had not worried him very much. In addition to his fever, he had an enlarged spleen, and auscultatory evidence of mitral stenosis. Blood cultures failed to yield pathogens on three occasions. Blood taken shortly after his admission (approximately seven days after the onset) revealed antibodies at a titre of 1 in 512 or greater. Blood was inoculated into guinea-pigs, but no confirmation was obtained of the presence of *C. burneti*. He remained febrile for six days after admission, and subsequently rapidly returned to normal health. As he lives in another State, follow-up investigation has been inadequate.

These three last cases reflect different aspects of the problem of chronicity. In the first, there is unequivocal evidence of continuing infection for three months, at any rate, and it remains to be seen whether infection has, in fact, persisted beyond that. In the second case, we have been unable to confirm that he really did have "Q" fever in 1945. Nevertheless, the complement-fixation test, together with the probability that organisms were present in the heart valve, suggest that he had the condition at the time of death, and we may suspect that this had been present for at least the previous year. In the last case, there is no certain evidence that the second illness was indeed "Q" fever. However, a titre of that order has not been found previously six years after an original infection, although residual titres up to 1 in 32 are not rare. The possibilities appear to be that this is either a flare-up of a chronic or latent infection, or a genuine reinfection, in both of which an early rise in titre would be understandable. In view of the high antibody titre, inability to isolate the organism cannot be taken as evidence against persistent infection. It is curious that in this case, also, there was evidence of valvular disease of the heart.

DISCUSSION

The chief aspect in which this series differs from other reported series of "Q" fever is in the incidence of pneumonia. Whilst it is true that no cases of pneumonia occurred in Derrick's original series, this has always been open to the criticism of inadequate X-ray control. However, even in this series, in which chest radiography was employed as a routine, only three patients had pneumonic changes, and one may wonder at the reason for this geographical disparity. One factor may be a difference in the manner of infection, but it is difficult to see that this is so. Moreover, respiratory, as distinct from pneumonic, involvement is not uncommon, as shown by the number of patients who developed acute bronchitis. It may be that the local strain of *C. burneti* is less virulent than overseas strains, or lacks their invasive power. Certainly, it is not lethal to guinea-pigs here, as it is elsewhere.

For the remainder, the generalized nature of "Q" fever is once again demonstrated. Too much should not be read into the electrocardiographic abnormalities. These prove only that "Q" fever is but another of the many infective processes in which transient myocardiopathy occurs. On the other hand, liver damage is frequent, as will be shown in anothe paper.

The importance of a protracted or chronic form of the disease cannot be emphasized too strongly, and several of the cases illustrate this aspect. A persistently increased erythrocyte sedimentation rate is usually, but not invariably, a feature of this. Proof of persistent infection by isolation of the organism from blood and urine is frequently unsuccessful, but a continuing high titre of complement-fixing antibodies may be suggestive. It is suggested that "Q" fever be considered in any case of apparent bacterial endocarditis in which blood culture fails to yield a growth of pathogens.

Nothing has been said of treatment. Physicians in this city are generally inclined to doubt the effectiveness of specific therapy. Most of the series were, in fact, untreated. An occasional patient received one or other of the broad-spectrum antibiotics, generally in an inadequate dosage. An attempt was made to evaluate the effect of chloramphenicol at a dose of two grammes *per diem* in a small series of patients who were admitted to hospital in the first five days of the disease. There was no difference in the duration of fever, as compared with alternate similar patients used as controls, but a loading dose was not given. In a condition which is usually short-lived, and in which patients often do not report until late, it is

difficult to assess the effect of therapy; but clearly a properly-controlled trial is badly needed, duration of fever, incidence of complications and persistence of an elevated erythrocyte sedimentation rate being used as evidence of the effect of treatment.

Finally, sufficient evidence has been given to indicate the economic importance of this condition in the meat industry. It cannot be said that this series is entirely unselected. For example, it may be that only those who are more ill reach hospital. To decide this, it would be necessary to compare the amount of time lost from work in this series with that lost by a similar group treated at home by their local practitioners. Regardless of this, however, it is evident that a very large amount of working time is being lost each year owing to "Q" fever.

ACKNOWLEDGEMENTS

Dr. E. H. Derrick suggested that I should undertake this survey, and I am grateful to him for this suggestion and for much valuable advice. I must acknowledge my indebtedness to the senior physicians of this hospital for agreeing to patients with suspected "Q" fever being admitted under my care, and in particular to Dr. H. G. Wilson for permission to report the case of W.McC. I am particularly appreciative of the encouragement and assistance given to me by Dr. J. I. Tonge, of the Laboratory of Microbiology and Pathology. Finally, I must thank Mr. N. Tingle and Mr. H. Thompson, of the technical staff of the laboratory, for their interest, and Mr. L. Godwin for preparing the illustrations.

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DIVIDENDS FROM MITRAL VALVOTOMY : A TWO TO SEVEN YEARS' FOLLOW-UP[1]

B. M. LEAROYD,[2] R. B. BLACKET,[3] B. C. SINCLAIR-SMITH,[3] F. H. MILLS,[4] J. H. HALLIDAY[5] and J. K. MADDOX[5]

From the Hallstrom Institute of Cardiology, Royal Prince Alfred Hospital, Sydney

SUMMARY

Studies have been made of 87 patients treated by finger-fracture valvotomy for mitral stenosis five or more years ago, and of 224 patients followed for only two years. At the time of operation the mean age of the patients was 38 years, and 34% had atrial fibrillation.

The functional classification of the patients and the results of the operation in each group are presented. The operative mortality was 5%, but ranged from 2·5% in Class II patients to 25% in Class IV patients. When the total number operated on is used as the base line, 61% of patients had a good result (improvement by at least one functional class) at two years. At five years this figure had fallen to 51%. The best results were obtained in the less severe cases.

Age in itself did not materially affect the outcome, but atrial fibrillation, whose incidence increased with age, lessened the prospects of success. Considerable cardiac enlargement was also an adverse factor.

Mitral incompetence present before operation or produced by surgery had an unfavourable effect on the outcome, and severe incompetence proved lethal. When moderate incompetence was present after operation, roughly two-thirds of the patients had a good result at two years, but this figure fell to one-third by five years.

The most significant factor in continued post-operative improvement was the completeness of separation of the adherent valve cusps. Division of one commissure gave temporary benefit to many patients, but by five years only one in four maintained their improvement. Deterioration was due to restenosis, which may also occur even when both commissures are adequately split.

We conclude that mitral valvotomy as originally practised leaves much to be desired. The pathology of advanced mitral stenosis does not readily lend itself to correction. We advise surgical treatment early in the disease, when disability is first noticeable and atrial fibrillation, embolism and valvular distortion have not occurred.

The results of more recent surgical techniques, although initially encouraging, remain to be evaluated.

SINCE its introduction by Bailey (1949) and Baker *et alii* (1950), mitral valvotomy has become the keystone of treatment in mitral stenosis. Indeed, the physician's chief responsibility is to choose the most appropriate time for surgical treatment; he must be well acquainted with the natural history of the disease, the mortality of the operation and the probability of improvement after surgery.

In this paper we present the clinical results at two and five years in all adequately documented public hospital cases in which operation was performed before the end of 1956. We have also endeavoured to ascertain those factors which are relevant to the prognosis.

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[2] Registrar.

[3] Honorary Assistant Physician.

[4] Honorary Surgeon.

[5] Honorary Physician.

METHOD

The pre-operative and follow-up data were obtained from case records made when the patients attended out-patient clinics before and after surgery. Each patient included in the survey was examined at least twice in the first twelve months and at yearly intervals after this. The assessment of a result was made purely on the basis of the patient's functional state, irrespective of the clinical signs. The results were analysed after the end of 1958. Five of the 87 patients operated on before the end of 1953 did not return for follow-up, so that only 82 have been included in the five-year analyses. For similar reasons, only 190 of the 224 patients operated on by the end of 1956 have been included in the two-year survey (Table I).

We believe that among those patients not followed up there was a higher proportion of good results than among those who continued

to report, as many of the former came from long distances and did not return because they were well.

A result was considered to be either "good" or "bad." A good result was one in which the patient's functional class was improved by at least one grade at the time when the analysis was made. A bad result included all other cases—that is, condition unchanged, condition worse, late deaths and operative deaths. By including operative deaths in the follow-up statistics, an accurate assessment of the overall prognosis from before operation can be made.

TABLE I
Number of Patients Followed Up

Period	Total Number of Operations	Operative Deaths	Untraced Patients	Patients Followed Up
1951 to 1953 ..	87	8	5	74
1951 to 1956 ..	224	19	34	171

PRE-OPERATIVE STATE OF PATIENTS

Functional Class

We classified our cases according to the pre-operative symptomatology, into the four grades of severity recommended by the New York Heart Association (Table II). At follow up they were similarly graded on a symptomatic basis, and for the purposes of this paper clinical signs have been ignored.

TABLE II
Classification of Patients

Disability	Grading	Numbers (and Percentage) in Follow-Up Investigation	
		At Two Years	At Five Years
Nil	Class I	—	—
Breathless on steps	Class II	101 (53%)	27 (33%)
Breathless on flat	Class III	76 (40%)	45 (55%)
Breathless at rest	Class IV	13 (7%)	10 (12%)
Total ..	.. ..	190 (100%)	82 (100%)

Some amplification of the classification of these cases is necessary for a clear assessment of the results. There were no asymptomatic patients (Class I). Class II patients were those who found the heavier housework—for example, scrubbing and polishing—to be too much for them. They were considerably distressed by sweeping and walking up steps. They frequently suffered from orthopnœa and attacks of pulmonary œdema. Class III disability reduced patients to the life of an invalid, so that they could only walk slowly on the flat and undertake no physical activity at all. They had often suffered with episodes of congestive cardiac failure, and usually required digoxin and diuretics to keep them free from œdema. With Class IV disability a patient was virtually confined to bed. It would seem that we have tended to grade our patients lower than most other authors (for example Ellis *et alii*, 1959), so that many of our Class III patients would be called Class IV and many of our Class II patients be called Class III in other clinics.

Age and Sex Distribution

There was the usual female preponderance in all age groups, of nearly 4 to 1. The mean age was 38 years at operation, with a range from 14 to 66 years.

Rhythm

Atrial fibrillation was present in 34% of the whole series, and its incidence increased progressively with each decade, from nil in patients aged less than 20 years to 60% in those aged over 40 years.

Embolism

Of the patients 16% had experienced at least one embolic episode before operation, and two-thirds of these had fibrillation at the time of surgery, which is twice the incidence in the series as a whole. Thus fibrillation predisposes to thrombosis and embolism, but is by no means the only factor causing these complications.

MORTALITY

As all the patients who died are well documented, the mortality rate has been calculated from the first 400 patients (public and private), although many of these are not included in the follow-up. In an attempt to relate mortality to pre-operative disability, it has been assumed that the functional classes of the first 400 patients were distributed in the same ratio as those followed. The mortality rates and causes of death for each class, calculated accordingly, appear in Table III.

Twenty patients died as a direct result of the operation, either on the table, or whilst still in hospital after surgery.

This is an overall mortality rate of 5%; but in Class II patients the mortality rate was 2·5%, and it rose to 25% in Class IV. These figures are comparable with Ellis and Harken's (1955)

mortality rate of 12% in their first 500 cases, which dropped to 5·2% in their second 500. Their mortality rate in Class II is only 0·8%, but Class IV still carried a mortality of 19% (Ellis, *et alii* 1959)

Since the end of 1956, approximately 150 valvotomies have been performed in this clinic with three operative deaths, a mortality rate of 2%. Two deaths were those of Class IV patients with considerable associated incompetence, and one was of a Class II patient.

Causes of Operative Death

From Table III it can be seen that four patients died on the operating table with cardiac arrest or ventricular fibrillation. These were all late cases, and the stenoses were very tight and extremely difficult for the surgeon to split. In seven cases severe mitral incompetence was produced by the operation, and these patients went into progressive heart failure after surgery and soon died. Five of them had grossly calcified valves and five had significant incompetence before surgery. Of the three embolic deaths, two were due to dislodgement of an atrial clot resulting in cerebral embolism.

TABLE III
Mortality Rate in the First 400 Cases

Cause of Death	Number of Deaths	Disability (Class)		
		II	III	IV
Cardiac arrest or fibrillation on operating table	4	—	3	1
Myocardial infection ..	1	—	1	—
Mitral incompetence ..	7	1	3	3
Embolism	3	2	1	—
Hæmorrhage	3	2	—	1
Cerebral vein thrombosis	1	1	—	—
Anæsthetic death (chronic pulmonary fibrosis) ..	1	—	1	—
Total	20	6	9	5
Mortality rate	5%	2·5%	6·4%	25%

The other was caused by a piece of calcium breaking off from the mitral valve and lodging in the left coronary artery. Three other patients died from blood loss, either immediately from an atrial tear, or in the post-operative phase. Of the three remaining deaths, one was due to myocardial infection and one was attributed to a cerebral venous thrombosis. The other patient died during the induction of anæsthesia; at autopsy this patient was shown to have diffuse interstitial fibrosis of the lung with insignificant mitral stenosis—a diagnostic error.

Overall Follow-Up

The clinical results at two and at five years are set out in Table IV. It can be seen that 72% of Class II patients had good results at two years and also at five years (73%). However, in Classes III and IV, not only is there a much lower proportion of good results at two years—48%—but there also seems to be an ominous deterioration by five years (39%), although these figures are not quite

TABLE IV
Overall Results

Class	At Two Years		At Five Years	
	Good	Bad	Good	Bad
II ..	73 (72%)	28 (28%)	20 (73%)	7 (27%)
III ..	41 (54%)	35 (46%)	20 (45%)	25 (55%)
IV ..	2 (15%)	11 (85%)	2 (20%)	8 (80%)
III and IV	43 (48%)	46 (52%)	22 (39%)	33 (61%)
Total	116 (61%)	74 (39%)	42 (51%)	40 (49%)

statistically significant. The difference between Class II and Classes III and IV both at two years and at five years is, however, highly significant ($P<0·01$). Clearly finger-fracture valvotomy as originally performed is much less successful in advanced cases, particularly in Class IV patients, of whom no more than 20% benefited from the operation. The bad results include 11 late deaths, nine of which were due to progressive cardiac failure, one to a late embolus and one to bacterial (infective) endocarditis. Two other patients have died after the five-year follow-up from unrelated causes—carcinoma of the lung and ruptured peptic ulcer. They both had good results at five years.

Specific Factors which May Influence Prognosis

Age

Both at two years and five years, the younger patients appeared to do better than the older ones. In neither group, however, were the figures significant at the 5% level. Age in itself should not weigh heavily in the pre-operative assessment.

Atrial Fibrillation

Fibrillation had an unfavourable effect on the outcome. Only 36% of patients with fibrillation achieved a good result at two years, as against 76% of those in normal sinus rhythm

($P=0{\cdot}001$). Not enough patients with fibrillation were followed for five years to allow any conclusions to be drawn from this group.

One-fifth of those in sinus rhythm developed fibrillation as a direct result of operation. In one in four of these patients sinus rhythm could not be restored. Thus, overall, 1 in 20 (5%) were precipitated into permanent fibrillation. This high incidence of fibrillation, either during the procedure or soon afterwards, has made it common practice for all patients to be digitalized before surgery, so that rapid, uncontrolled fibrillation does not become an additional hazard in the early post-operative phase. Attempts should always be made to cause these patients to revert to normal sinus rhythm. In our experience this is most likely to succeed after the second or third week. It has not been the custom to give anticoagulant drugs to these patients prior to the attempt at reversion. Usually we do not try to cause reversion in patients with established fibrillation, although this was once achieved in a man known to have had fibrillation for five years.

Embolism

Thirty patients (16%) had experienced at least one embolic episode before operation, and one-third of these had suffered more than one. Patients with pure stenosis and those with mixed lesions were equally prone to embolic episodes. Of those who had emboli, two-thirds had fibrillation at the time of surgery, and in 50% of these the surgeon found a clot in the atrium. Atrial thrombosis is therefore episodic in many cases.

Of those patients who had suffered from embolism before operation, one in eight (13%) had a further episode on the operating table. This is twice the incidence of operative embolization in patients who gave no history of previous embolism. However, this risk must be accepted, for once a patient has had an embolus, he is even more likely to have another one if left untreated (33% chance in this series). The risk of operative embolism, though considerable (13%), is less than the risk of leaving mitral stenosis untreated. We have accepted embolism as an indication for surgery regardless of the severity of the stenosis. In fact, in all these cases the stenosis has been moderate or severe, and this in itself has warranted valvotomy. There is much to be said for a preliminary course of anticoagulant therapy to prevent embolism during surgery. These patients have no greater risk of a late embolus after operation than do other patients, the incidence being about 2·5% in this series.

Operative embolism is generally attributed to an atrial clot being dislodged by the surgeon's finger. Of 17 cases of operative embolism (not all in this series), a clot was felt in only four; but the valve was heavily calcified in nine, and neither calcium nor clot was reported in the other four. Interestingly enough, 11 of these 17 patients were in sinus rhythm—a higher incidence than expected. It would seem that a calcified valve is perhaps a more frequent cause of operative embolism than atrial clot. Although clot is more commonly present, the surgeon is able, by various technical manœuvres, to reduce the likelihood of its dislodgement into the systemic circulation. There is little he can do to prevent dissemination of calcareous débris from a tough, craggy valve. It is not without significance that of the three embolic deaths, at least one was due to a calcific embolus which lodged in the coronary circulation.

X-Ray Findings

Table V sets out the results at the end of two years and five years in relation to the pre-operative cardio-thoracic ratio. Nearly half the patients (47%) had moderately enlarged hearts (cardio-thoracic ratio 0·51 to 0·57), 23% had ratios below 0·50, and 30% had ratios in excess of 0·57.

TABLE V

Results in Relation to the Cardio-Thoracic Ratio Before Operation

Pre-operative Cardio-Thoracic Ratio	Result			
	At Two Years		At Five Years	
	Good	Bad	Good	Bad
Less than 0·51 ..	27 (64%)	15 (36%)	9 (69%)	4 (31%)
0·51–0·57	59 (69%)	27 (31%)	19 (50%)	19 (50%)
More than 0·57 ..	30 (50%)	30 (50%)	10 (37%)	17 (63%)

In general terms, it mattered little whether a heart was normal in size or moderately enlarged; a good result could be expected in about two-thirds of cases (64% to 69%) in both groups. The larger hearts (cardio-thoracic ratio $>0{\cdot}57$) carried a lower success rate (50%), but with the small number involved, the difference was not statistically significant.

In the two to five years' period there was deterioration in those with moderately and markedly enlarged hearts. These results also are not statistically significant, in view of the small numbers. Insufficient patients with normal heart size before operation have been

followed for five years to allow any conclusion to be drawn for this group. We believe that, whilst a small heart favours a good result, a large heart does not preclude it.

Table VI shows the results in relation to the alteration in cardiac size (change in cardio-thoracic ratio in excess of 0·02) produced by the operation. The post-operative films were taken mostly within the first year. As expected, those whose hearts had diminished in size got the best results—90% were in the "good" category at two years and 72% at five years. Patients whose heart size remained unchanged had a success rate of only 48% at five years, compared with 29% of those whose hearts increased in size after valvotomy. Clearly post-operative cardiac size is of importance in the prognosis, and this may be related to the difficulty and adequacy of the valvotomy. It is seen from Table VIII that in 81% of patients with "normal" heart size a two-finger or greater split of the valve was obtained, whilst in only 43% of those with the largest hearts was such a split obtained.

TABLE VI

Results in Relation to Alteration in Heart Size After Surgery

Change in Cardio-Thoracic Ratio	Result			
	At Two Years		At Five Years	
	Good	Bad	Good	Bad
Reduced by more than 0·02 ..	27 (90%)	3 (10%)	8 (72%)	3 (28%)
Unchanged ..	43 (72%)	17 (28%)	12 (48%)	13 (52%)
Enlarged by more than 0·02 ..	19 (44%)	24 (56%)	4 (29%)	10 (71%)

Hæmodynamic Findings

Cardiac catheterization was commonly used in the assessment of patients with mitral stenosis in the early days of mitral valvotomy. We have correlated the results of surgery with the catheterization findings in 117 cases. As in most other clinics, we began by catheterizing the hearts of all candidates for surgery, but now use the procedure infrequently. The results obtained at catheterization and the operative result in these cases appear in Table VII. Pulmonary artery pressure, pulmonary vascular resistance, right atrial pressure and cardiac output at rest and after standard recumbent leg exercise were examined. For each parameter the patients were divided into three groups.

TABLE VII

Results in Relation to Catheterization Findings

Table VIIA

Result	Mean Pulmonary Artery Pressure		
	Less than 20 mm. Hg	20 to 40 mm. Hg	40 mm. Hg or More
At two years:			
Good ..	5	28	47
Bad ..	4	21	17
At five years:			
Good ..	1	4	18
Bad ..	2	13	12

Table VIIB

Result	Pulmonary Vascular Resistance		
	Less than 3 Units	3 to 8 Units	More than 8 Units
At two years:			
Good ..	22	25	20
Bad	13	21	8
At five years:			
Good ..	4	9	11
Bad	7	15	4

Table VIIC

Result	Resting Cardiac Output		
	More than 4·5 Litres per Minute	3·0 to 4·5 Litres per Minute	Less than 3·0 Litres per Minute
At two years:			
Good ..	20	28	19
Bad	6	24	9
At five years:			
Good ..	7	6	12
Bad	4	14	5

Table VIID

Result	Cardiac Output after Exercise		
	More than 6·0 Litres per Minute	4·0 to 6·0 Litres per Minute	Less than 4·0 Litres per Minute
At two years:			
Good ..	10	12	18
Bad	7	8	10
At five years:			
Good ..	5	4	14
Bad	4	9	6

Table VIIE

Result	Mean Right Atrial Pressure	
	Less than 4 mm. Hg	More than 4 mm. Hg
At two years:		
Good	39	23
Bad	21	15
At five years:		
Good	9	14
Bad	14	7

Patients with normal or moderately elevated mean pulmonary artery pressure (<40 mm. Hg) and pulmonary vascular resistance showed similar results at two years, and both groups showed similar deterioration at the five-year follow-up. Those patients with mean pulmonary artery pressures in excess of 40 mm. Hg and pulmonary resistances in excess of eight Wood units were considerably better at two years and maintained their improvement in the five-year follow up. These results are statistically significant.

The high-pressure, high-resistance group differed from the moderate group (20 to 40 mm. pressure, 3 to 8 units of resistance) in several ways. In it were only 20% with fibrillation,

TABLE VIII

Relation of Split Achieved to Heart Size, Pulmonary Artery Pressure and Pulmonary Vascular Resistance

	Split Achieved	
	Less than Two Fingers	Two Fingers or More
Cardio-thoracic ratio:		
Less than 0·51	6 (19%)	26 (81%)
0·51 to 0·57	26 (38%)	43 (62%)
More than 0·57	27 (57%)	20 (43%)
Mean pulmonary artery pressure:		
Less than 20 mm. Hg ..	5 (50%)	5 (50%)
20 to 40 mm. Hg	17 (43%)	23 (57%)
More than 40 mm. Hg ..	21 (46%)	25 (54%)
Pulmonary vascular resistance:		
Less than 3 units	16 (46%)	19 (54%)
3 to 8 units	18 (45%)	22 (55%)
More than 8 units	12 (57%)	9 (43%)

as against 35% in the moderate group; it had 92% with right ventricular hypertrophy, judged electrocardiographically, as against 62%; and it had only 50% with cardiac enlargement (cardio-thoracic ratio in excess of 0·50), as against 66% of the moderate group. On the other hand, there was no great difference in the tightness of the stenosis—if anything, the patients with severe pulmonary hypertension had slightly smaller valve orifices. As Table VIII shows, the size of the split achieved was the same in the high-resistance cases as in the others.

Table VII shows that a knowledge of the cardiac output at rest or on exercise is of no value in predicting the outcome of surgery. Both at two years and at five years, patients with a resting output of less than 3 litres a minute have as much chance of being improved as those with outputs in excess of 4·5 litres a minute. Curiously enough, those with intermediate outputs (3·0 to 4·5 litres a minute) did rather worse than the remainder. The response to exercise was equally unhelpful, improvement occurring in as many with fixed outputs as in those with normal or near normal rises in output on exercise. Despite the fact that Class IV patients with chronic congestive heart failure did badly, this was not apparent in a comparison of the right atrial pressure and operative result. At two years, patients with mean pressures above 4 mm. Hg did just as well as those with pressures below this figure. At five years the group with the higher pressure appeared to do better, but the numbers were small. This paradoxical result is perhaps explained by our custom of catheterizing the hearts of candidates for valvotomy after the maximum benefit has been obtained from bed rest, digitalis and diuretics. The mean right atrial pressure alone is then less likely to differentiate the good from the bad myocardium.

The hæmodynamic grouping of our results emphasizes the importance of a powerful myocardium in capitalizing on a mitral valvotomy. Patients with high pulmonary resistance, normal rhythm and evidence of right-sided hypertrophy have shown a clear superiority in the post-operative result over those with lower resistances, a more dilated heart and atrial fibrillation.

Mitral Incompetence

It is generally agreed that associated mitral incompetence is a relative contraindication to surgery, depending, of course, on its severity. The reasons for this are twofold. Firstly, severe degrees of stenosis and incompetence are mutually exclusive lesions. A tightly stenosed valve cannot transmit a significantly incompetent jet, and conversely, a severe degree of incompetence indicates that the valve orifice cannot be greatly stenosed. Secondly, splitting of an incompetent valve usually tends to increase the amount of regurgitation out of proportion to the relief of the stenosis. Although the clinical data will usually point to the dominant lesion, this is not absolutely reliable, and mistakes will occur in both directions. In the 190 patients followed for two years, there were four whose incompetence had been over-estimated and 23 whose incompetence had been underestimated by the physician prior to surgery. In three other cases neither stenosis nor incompetence was significant.

The results at two years and five years for the patients with varying degrees of incompetence before operation are set out in Table IX. In the five-year follow-up there were seven patients in the total of 82 who had significant or dominant incompetence; two of these got a good result. In the later patients followed for only two years, 32 out of 190 (17%, or

approximately double the proportion in the five-year follow-up) had significant incompetence. This reflected our natural desire to help those whose prognosis without surgery seemed poor. Such liberalization of the indications for surgery did not pay dividends, for only seven, or 22%, of the 32 showed improvement at two years. By contrast, slight incompetence with stenosis, as assessed clinically, yielded the same success rate as pure stenosis.

The creation of incompetence at operation is also undesirable, and the adverse effect on the prognosis can be deduced from Table X. Both at two years and five years, severe incompetence after operation was invariably associated with a bad prognosis. Moderate incompetence had no appreciable overall effect at two years, but in the five-year follow-up only 35% of patients with this lesion continued to do well. That mitral incompetence may take some years to show its ill effects was also found by Ellis *et alii* (1959).

TABLE IX

Results in Relation to Degree of Incompetence Present Before Operation

Degree of Incompetence Present Before Surgery	Result			
	At Two Years		At Five Years	
	Good	Bad	Good	Bad
Nil: pure stenosis	85 (68%)	36 (32%)	28 (52%)	26 (48%)
Slight: with dominant stenosis	24 (65%)	13 (35%)	12 (57%)	9 (43%)
Moderate: mixed lesions	7 (22%)	25 (78%)	2 (29%)	5 (71%)

Of the 74 patients who had not done well at the two-year follow-up, 44, or 60%, had moderate or marked incompetence. Incompetence, either present before operation or produced as a result of the operation, clearly contributed in large measure to the bad results at two years. Yet of the 40 patients with bad results at five years, only 18, or 45%, had serious incompetence. Clearly, other factors were concerned in the failure to maintain improvement after two years; in particular, the adequacy with which the surgeon relieved the stenosis must be of interest.

TABLE X

Results in Relation to Degree of Incompetence Present After Operation

Degree of Incompetence Present after Surgery	Result			
	At Two Years		At Five Years	
	Good	Bad	Good	Bad
Nil and slight ..	79 (72%)	30 (28%)	36 (62%)	22 (38%)
Moderate ..	37 (62%)	23 (38%)	6 (35%)	11 (65%)
Marked	—	21 (100%)	—	7 (100%)

Adequacy of Surgery

The results have therefore been correlated with the split achieved by the surgeon. Cases have been separated into two groups, those in which at least a two-finger split was achieved and those in which it was less (Table XI).

At two years, those patients with two-finger splits had 74% of good results, and those with less than two-finger splits had 63% of good results. This difference is not statistically significant. At five years, however, those patients who had good splits maintained their improvement (74% of good results), but those who had poor splits had only 28% of good results ($P<0.01$). Adequacy of the split is obviously critical for the continued success of the operation.

TABLE XI

Results in Relation to Split Achieved

Class	Result							
	At Two Years				At Five Years			
	Less than Two Fingers		Two Fingers or More		Less than Two Fingers		Two Fingers or More	
	Good	Bad	Good	Bad	Good	Bad	Good	Bad
II	17	6	47	15	—	3	18	4
III	20	10	19	6	5	9	8	4
IV	1	6	—	2	1	3	—	1
Total ..	38 (63%)	22 (37%)	66 (74%)	23 (26%)	6 (28%)	15 (72%)	26 (74%)	9 (26%)

Table XI shows the distribution of adequate valvotomy within the functional classes. By adding the "good" and "bad" in each class horizontally, it will be seen that the proportion of satisfactory splits is much higher in Class II (73%) than in either Class III (45%) or, particularly, Class IV (22%). Also, in Classes II and III, relief of symptoms at two years can be expected even though the valvotomy is not complete; but by five years the effects of an inadequate valvotomy emerge in all groups.

Atrial fibrillation does not weigh heavily against adequate surgery. Nevertheless, the results at two years were less satisfactory in patients with fibrillation, which possibly indicates an adverse myocardial factor in these cases.

DISCUSSION

The results obtained by finger-fracture valvotomy (valvuloplasty, commissurotomy) in this clinic are very similar to those reported elsewhere (Glover *et alii*, 1955; F. H. Ellis *et alii*, 1958; Likoff and Uricchio, 1958; L. B. Ellis *et alii*, 1959). Our own figures were calculated from the pre-operative totals, and thus include operative mortality in the follow-up of the survivors. This seems to us a legitimate approach to the assessment of any procedure; but it does lower the apparent success rate of the survivors, particularly in the more severely ill patients, whose mortality rate was highest.

The superiority of operative over medical management in mitral stenosis has been shown convincingly in the excellent report of L. B. Ellis *et alii* (1959). They compared their results with those of a medically treated series, and the survival after surgery was far superior. Surgery offers a longer life and a more active one for the great majority of patients. With better selection of patients and increasing surgical experience, the mortality rate from operation has fallen steadily, and our present rate of 2%, while not the best reported, is almost as low as is possible if the most difficult cases are not refused. However, unexpected hazards, such as embolism and infection with antibiotic-resistant organisms, will continue to crop up and mar the record even in the less severe cases.

The present results reinforce recent experience (Bailey *et alii*, 1957) that in many cases, particularly with advanced lesions, finger-fracture valvotomy does not offer lasting or even temporary relief. This remains true even in the more recent cases in this series, in which surgical experience has been greater. They do show, however, that partial relief of stenosis may offer a short-term respite, particularly for patients with the lesser degrees of incapacity.

The poor results obtained in advanced disease with atrial fibrillation and cardiac enlargement emphasize that the physician has no reason to delay surgery once symptoms have appeared. All physicians of experience have seen the unhappy results of procrastination—particularly, disabling cerebral embolism—in patients who were only moderately handicapped. Indeed, this is one situation in which a physician should encourage the waverers to undergo surgery while they are still relatively fit.

In their long-term studies, the Mayo Clinic group (Ellis *et alii*, 1958) and the Boston group (Ellis *et alii*, 1959) also found that atrial fibrillation and advancing age lessened the probability of success. As our results show, atrial fibrillation is a function of age; 60% of patients aged over 40 years with mitral stenosis have fibrillation. It is well known that atrial fibrillation in itself may cause cardiac disability in clinically normal hearts (Phillips and Levine, 1949). In patients with mitral stenosis it lowers the cardiac output (Blacket *et alii*, 1953) and in a small proportion of patients with mild mitral stenosis it is associated with recurrent heart failure (Fleming and Wood, 1959). That this may be due to a myocardial factor was appreciated at least 10 years ago by Cournand and his group (Ferrer *et alii*, 1955; Harvey *et alii*, 1955), who found hæmodynamic evidence of myocardial dysfunction in about 10% of patients with mitral valve disease. Although Fleming and Wood found myocardial dysfunction the dominant factor in only 3·2% of their 750 patients, they do not exclude the possibility that it may contribute to disability in others with more severe stenosis.

The converse proposition, that myocardial hyperfunction (as shown by a high pulmonary artery pressure and a strong right ventricle) may make for good results, was suggested by L. B. Ellis and his colleagues and is strongly supported by the present findings. As far as we could tell from the records, our very hypertensive patients, as a group, had no more adequate valvotomies than the remainder, but obtained a better result. In contrast, those with atrial fibrillation but without extreme hypertension obtained, on the whole, a less adequate valvotomy which undoubtedly contributed in part to their less satisfactory result.

The present findings show very well the importance in the long term of an adequate valvotomy where both commissures are split. They also show that mitral incompetence after surgery materially lessens the prospects of success, and if moderate or extreme may lead to deterioration and death after operation. For the best results from the operation, the

physician must choose patients with pure or almost pure stenosis, and the surgeon must split both commissures to the annulus without producing incompetence. Early in their experience, surgeons were well aware of the danger of incompetence, but not of the necessity to split the commissures completely. The difficulty of obtaining an adequate split in tough and calcified valves often dissuaded them from tackling the less accessible posteromedial commissure for fear of producing incompetence. Because of this, we saw many patients in whom the auscultatory signs of mitral stenosis persisted unchanged after surgery. Some 70% of these patients showed improvement at two years, but in only 28% has improvement continued for five years or more. Three years after the operation was begun, when the first 50 patients had been followed for two years, the results seemed reasonable. It was only after five years that the disappointing overall results with splitting of one commissure became apparent.

There is evidence both here and elsewhere that inadequately split valves become restenosed (Bailey *et alii*, 1957). Without hæmodynamic studies, it is impossible for us to estimate the frequency of this variety of restenosis. However, we have already submitted 11 patients to a second operation, of whom nine had had incomplete first operations. That two patients with an adequate commissurotomy also suffered restenosis is disappointing, but is in accord with experience elsewhere (Bailey *et alii*, 1957; Belcher, 1958; Patterson and Marshall, 1959). It demonstrates that the abnormality itself may continue to provide an obstacle to permanent cure even with more adequate operations. There is no doubt in our minds that new approaches to the mitral valve, either through the right atrium (Bailey and Morse, 1957) or through the left atrium and ventricle together, achieve a more satisfactory result as judged by auscultation in the postoperative period. The dual left atrial-left ventricular approach has been used here now for about 12 months, but we are already seeing patients with residual signs in whom the surgeon reported complete splitting. Other patients have developed mitral incompetence. Clearly, the results of these new approaches should be better; but how much better they really are, remains to be seen.

As in the series of L. B. Ellis and his colleagues (1959), mitral incompetence has played an important part in deterioration after operation. They found that deterioration due to mitral incompetence was greater at five years than at one year, and stated that the adverse effects of incompetence frequently took some time to develop. They also found that at one year the greatest difference in results occurred between those with moderate and those with severe incompetence. Our results accord with theirs. Severe incompetence is obviously lethal; moderate incompetence may be endured, but usually not for many years.

Rheumatic fever recurred in 38 of 228 patients in the Boston series, whose condition deteriorated after initial improvement. This cause of deterioration was not mentioned by the Mayo group, and it has not been a feature of our material as far as we know. In view of the rarity of active rheumatic fever as judged clinically in adult patients in the fourth and fifth decades who have not undergone operation, the Boston experience is surprising. Nonetheless, the number with recurrent rheumatism still represents a small percentage of the 1000 patients studied by Ellis and his colleagues.

The value of surgery in preventing recurrent embolism has been amply demonstrated in our own material, and is confirmed by the Boston and Mayo groups. Although it may be argued that embolism itself is not an indication for surgery, as it may occur with minimal stenosis, we have found that the stenosis is usually severe enough to merit intervention.

The lack of correlation between hæmodynamic findings at catheterization and the success of the operation surprised us. As we mentioned above, Harvey and her colleagues have shown that myocardial insufficiency is not an uncommon complication of mitral stenosis, and almost certainly plays some part, or even the dominant part, in a few patients who do not do well after surgery. However, the results of adequate valvotomy do show that patients with a low fixed cardiac output frequently improve tremendously, and the evidence for myocardial failure must be overwhelming before surgery is contraindicated.

Now that the transvalvular gradient can be determined directly by left atrial puncture (Allison and Linden, 1953) and the flow measured by dye-dilution techniques, cardiac catheterization is seldom necessary in the selection of patients for operation. Clinical assessment is nearly always sufficient, and may be supplemented in doubtful cases with mixed lesions by left atrial puncture.

Finally, the present study emphasizes once again the importance of continual reassessment of the newer techniques in cardio-vascular surgery. At the outset, mitral valvotomy had an appealing simplicity about it which blinded many of us to its shortcomings. The passage

of time has shown that these are real enough, and that continued observation and care of patients with mitral stenosis will provide the stimulus for more satisfactory surgical treatment.

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THE RELATIONSHIP BETWEEN BLOOD FIBRINOLYTIC ACTIVITY, SERUM LIPOPROTEINS AND SERUM CHOLESTEROL IN ATHEROSCLEROTIC ARTERIAL DISEASE[1]

P. J. NESTEL[2]

From the University Department of Melbourne, Royal Melbourne Hospital

SUMMARY

Blood fibrinolytic activity, serum beta-alpha lipoprotein ratios and serum cholesterol levels have been compared in patients with myocardial infarction, and in patients with intermittent claudication. Fibrinolytic activity was significantly less in patients with intermittent claudication than in patients with myocardial infarction. Serum cholesterol levels and serum lipoprotein ratios were significantly higher in patients with intermittent claudication than in patients with myocardial infarction. The significance of these results is discussed.

Coefficients relating fibrinolytic time to serum cholesterol level and serum beta-alpha lipoprotein ratio were highly significant in patients with intermittent claudication, but not significant in patients with myocardial infarction. When the data from both groups were combined, the coefficients relating fibrinolytic time to serum lipoprotein ratios and to serum cholesterol levels were found to be highly significant. This suggested that the presence of a significant correlation between fibrinolysis and lipids depended on the inclusion of subjects whose fibrinolytic times and lipid levels were high.

THIS study was prompted by the observation that there was, on the average, less fibrinolytic activity in the blood of patients with occlusive arterial disease of the legs than in a group of control subjects (Nestel, 1959), and that the mean serum beta-alpha lipoprotein ratio of such patients was higher than that of a group of controls and also of a group of patients with ischæmic heart disease (Nestel, 1960).

Greig and Runde (1957) have shown that beta lipoproteins depress fibrinolysis *in vitro*, and Kwaan and MacFadzean (1957) have found that in rats fibrinolytic activity was depressed after they had been given cholesterol.

It seemed possible, therefore, that a relationship between fibrinolytic activity and serum lipids might exist in patients with occlusive vascular disease of the legs and perhaps in others, and a study was designed to test this possibility. Blood fibrinolytic activity, serum beta-alpha lipoprotein ratios (serum lipoprotein ratio) and serum cholesterol levels in groups of patients with intermittent claudication and with myocardial infarction are discussed here, and their interrelationships are compared.

MATERIAL AND METHODS

Two groups of patients were studied. Group I comprised 36 patients who had recovered from a myocardial infarction. Blood was obtained from them four to eight weeks after the infarction. By that time they had been discharged from hospital and were not being treated with anticoagulants. Their mean age was 52 years. Group II comprised 60 patients with intermittent claudication due solely to long-standing atherosclerotic occlusion. Evidence of coronary artery disease was present in some. Patients with diabetes mellitus were excluded. Their mean age was 60 years.

Fibrinolytic activity, serum lipoprotein ratio and serum cholesterol level were measured in each subject. Fibrinolytic activity was measured in diluted whole blood by the method of Fearnley *et alii* (1957), serum lipoproteins were separated electrophoretically and stained (Swahn, 1953), and serum cholesterol was measured by the method of Sackett (1925).

Correlation coefficients were calculated for serum cholesterol level, serum lipoprotein ratio and fibrinolytic time separately for each group of patients, and then on the combined data from both groups.

RESULTS

The values found for fibrinolytic time, serum lipoprotein ratio and serum cholesterol level in the two groups are shown in Table I.

The mean times taken for complete lysis to occur (which are inversely related to fibrinolytic activity) and the standard deviations were $11 \cdot 1 \pm 5 \cdot 69$ hours for the intermittent claudication group and $5 \cdot 8 \pm 3 \cdot 09$ hours for the

[1] Received on March 11, 1960.

[2] Assistant, Department of Medicine, University of Melbourne.

myocardial infarction group, and this difference was highly significant ($P<0{\cdot}001$). The mean fibrinolytic time for the myocardial infarction group did not differ significantly from that of a control group previously discussed (Nestel, 1959).

TABLE I

Fibrinolytic Time, Lipoprotein Ratio, and Serum Cholesterol Level of 36 Patients with Myocardial Infarction and 60 Patients with Intermittent Claudication[1]

Subjects	Fibrinolytic Time (Hours)	Lipoprotein Ratio	Serum Cholesterol Level (Milligrammes per 100 ml.)
Patients with myocardial infarction (36) ..	5·8±3·09	3·7±1·04	251±49
Patients with intermittent claudication (60) ..	11·1±5·69	4·8±2·90	310±64

[1] Figures express mean values and standard deviation.

The mean serum lipoprotein ratio for the intermittent claudication group (4·8±2·90) was significantly higher than that for the myocardial infarction group (3·7±1·04; $P<0{\cdot}001$). This is in agreement with a previous finding in two similar groups (Nestel, 1960).

The mean serum cholesterol levels were also significantly higher ($P<0{\cdot}001$) for the intermittent claudication group (309·6±63·56 mg. per 100 ml.) than for the myocardial infarction group (251·3±48·78 mg. per 100 ml.).

Table II shows the correlation coefficients and the levels of significance for the various comparisons in both groups. In patients with intermittent claudication, the coefficients relating fibrinolytic time to serum lipoprotein ratio and to serum cholesterol level, though not high, reached a high level of significance.

In patients with myocardial infarction, the coefficients relating fibrinolytic time to lipoprotein ratio and to serum cholesterol level were not significant.

The coefficients relating serum lipoprotein ratios to serum cholesterol level were highly significant for both groups.

When correlation coefficients were calculated for the combined data from both groups, a highly significant relationship was found between fibrinolytic time and serum lipoprotein ratio, and between fibrinolytic time and serum cholesterol level ($P<0{\cdot}001$), for both comparisons.

Figure I, in which the data from both groups have been combined, illustrates the relationship of fibrinolytic time to serum lipoprotein ratio and to serum cholesterol content at various levels. All the observations have been divided into five groups at intervals of 100 mg. per 100 ml. of serum for cholesterol and of two units for the lipoprotein ratio; the mean values with their standard errors and the number of observations are recorded for each of the five groups.

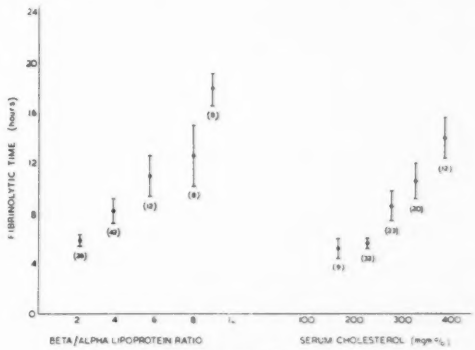

FIGURE I

The relationship of fibrinolytic time to serum beta-alpha lipoprotein ratio and to serum cholesterol level, calculated from observations on 60 patients with intermittent claudication and 36 patients with myocardial infarction. All the observations have been divided into five groups at intervals of 100 mg. per 100 ml. of cholesterol and of a ratio of two units of lipoprotein, and the mean value with their standard errors and the number of observations are shown

TABLE II

Correlation Coefficients and Levels of Significance for Fibrinolytic Time, Beta-Alpha Lipoprotein Ratio and Cholesterol Level in Patients with Intermittent Claudication and with Myocardial Infarction

Comparison	Patients with Intermittent Claudication			Patients with Myocardial Infarction		
	Number of Estimations.	Coefficient	Level of Significance	Number of Estimations	Coefficient	Level of Significance
Fibrinolytic time and beta-alpha lipoprotein ratio ..	60	+0·513	0·1%	36	+0·306	Not significant
Fibrinolytic time and cholesterol level	60	+0·427	0·1%	36	+0·327	Not significant
Cholesterol level and beta-alpha lipoprotein ratio ..	60	+0·348	1%	36	+0·611	0·1%

There appears to be a direct relationship between fibrinolytic time and serum lipoprotein ratio at all levels; but a relationship exists between fibrinolytic time and serum cholesterol content only at serum cholesterol levels above about 250 mg. per 100 ml.

DISCUSSION

It is clear that patients with intermittent claudication have significantly less blood fibrinolytic activity and higher serum cholesterol levels and higher serum lipoprotein ratios than patients with myocardial infarction. The possibility that there might be a simple relationship between fibrinolytic activity and serum lipids was supported by the highly significant correlation coefficients found in patients with intermittent claudication and in the combined data from both groups. However, the fact that no such correlations existed in patients with myocardial infarction suggested that the presence of a significant correlation between lipids and fibrinolysis depended on the inclusion of subjects whose lipid levels and fibrinolytic times were high. This might be true for serum cholesterol level, in which figures below about 250 mg. per 100 ml. appear to be unrelated to fibrinolytic time, whereas above that concentration a relationship does exist (Figure I); but this does not apply to serum lipoprotein ratios, which appear to be related to fibrinolytic times at all levels. These findings may be analogous to those of Goldrick (1960), who showed that a close correlation existed between whole-blood clotting time and beta lipoprotein cholesterol level when the mean values for these were high, but not when they were low.

The differences between the two groups are interesting, since the patients differed only in that their symptoms were produced by the occlusion of two separate arterial territories. This again raises the possibility, previously considered (Nestel, 1960), that these differences may reflect the operation of different factors influencing the development of occlusive arterial disease in different territories.

The differences in fibrinolytic activity between the two groups may be related to the greater extent of thrombosis in the arteries of the legs of the patients with intermittent claudication, since it is the extent of the thrombosis rather than the degree of atherosclerosis which distinguishes such patients from men of similar age without symptoms of intermittent claudication (Harrison, 1953). Extensive thrombosis is not a striking feature of myocardial infarction, and in about half such subjects which come to autopsy there is no evidence of recent coronary thrombosis (Bjerklund, 1957). The finding of normal fibrinolytic activity in patients several weeks after a myocardial infarction is in agreement with the observation of Hume (1958) and of Merskey *et alii* (1958).

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A Review Written by Request

RED-CELL METABOLISM: FUNDAMENTAL AND CLINICAL ASPECTS

G. C. DE GRUCHY[1]

From the University of Melbourne Department of Medicine, St. Vinvent's Hospital, Melbourne

UNTIL about 1940, the red cell was generally regarded as a more or less inert envelope containing hæmoglobin. However, studies on the biochemistry of the cell over the past 20 years have shown that the cell is in a state of continual metabolic activity, and that its structural and functional integrity is largely dependent on this metabolic activity. Denstedt (1953) has succinctly summarized present opinion as follows: "The red cell is now regarded as a living cell in the sense that it exists in a dynamic state, does work, and requires sustenance to maintain its energy metabolism."

The purpose of this review is twofold: (i) to give a short account of present knowledge of the fundamental aspects of red-cell metabolism; (ii) to indicate the nature and clinical importance of the metabolic defects which have recently been demonstrated in certain congenital hæmolytic anæmias. Particular emphasis is given to the genetically determined drug-induced hæmolytic anæmias, which are assuming increasing clinical importance in Australia because of the recent increase in the number of persons of Mediterranean origin, in whom there is a relatively high incidence of a metabolic defect predisposing them to this type of hæmolysis.

The metabolic changes occurring in blood stored for transfusion will not be discussed; details are given by Rappoport (1947), by Denstedt (1953), by Gabrio *et alii* (1956), and by Bartlett and Barnet (1960).

NORMAL RED-CELL METABOLISM

METABOLISM OF IMMATURE CELLS

The red cell undergoes very definite changes in metabolism as it matures from the early nucleated red cell through the reticulocyte stage to the mature red cell. The metabolism of the developing cells will be mentioned briefly before that of the mature cell is discussed.

The Nucleated Red Cell

The nucleated red cell appears to take part in the general metabolic reactions characteristic of nucleated cells of other tissues. Thus it synthesizes proteins, lipids and carbohydrates. Synthesis of deoxyribonucleic acid (DNA) occurs mainly in the nucleus, and of ribonucleic acid (RNA) mainly in the cytoplasm and nucleolus. The RNA is responsible for the basophilia of the cytoplasm. Synthesis of hæmoglobin from the pigmented moiety hæm and a colourless protein globin is a major function of the nucleated red cell. Hæm is synthesized in the cell through a series of intermediates from relatively simple precursors; the reactions leading to its synthesis are summarized by London (1960) and set out in detail by Rimington (1958). The change in colour of the ripening cytoplasm in stained films from deep blue to pink as the cell matures is due to the progressive formation of hæmoglobin and the simultaneous reduction of RNA synthesis (Thorell, 1947). A number of aspects of normoblast metabolism are reviewed by Lajtha (1957).

The Reticulocyte

Reticulocyte maturation is characterized by progressive loss of RNA, the transition to the mature cell being marked by disappearance of RNA. The reticulocyte is capable of hæmoglobin synthesis (Walsh *et alii*, 1949); it has been calculated that about 5% of the cell's hæmoglobin is synthesized in the reticulocyte, most of it while the reticulocyte is still in the marrow. Details of the enzyme activity of the reticulocyte are given by Rubenstein *et alii* (1956). Glycolysis is more active than in the mature cell, and as the enzymes of the Krebs tricarboxylic acid cycle are present, respiration

[1] The work from the writer's laboratory reported in this paper was aided by a grant from the National Health and Medical Research Council.

occurs at a much higher rate. Lowenstein (1959) reviews the biochemistry of the reticulocyte in detail.

METABOLISM OF THE MATURE RED CELL

The mature red cell differs significantly in its metabolism from the nucleated red cell and reticulocyte. It contains no RNA and thus does not synthesize protein ; there is no synthesis of hæmoglobin. Recent evidence suggests that there is little or no synthesis of lipid (Marks and Gellhorn, 1959). The enzymes of the Krebs tricarboxylic acid cycle are absent, and there is only a small consumption of oxygen. The chief metabolic activity of the cell is glycolysis, which provides the energy necessary for maintenance of the structural integrity of the cell.

Red-Cell Glycolysis

Glucose enters the cell from the plasma and is then metabolized by two pathways, the Embden-Meyerhof pathway and the pentose phosphate pathway. The first step in these two pathways is identical—that is, the conversion of glucose to glucose-6-phosphate, the reaction being catalysed by hexokinase and requiring ATP as a specific coenzyme (Figure II). It appears that, in normal cells, about 90% of the glucose is metabolized by the Embden-Meyerhof pathway (Murphy, 1960).

The Embden - Meyerhof Pathway. — The Embden-Meyerhof pathway is anaerobic, and results in the formation of lactic acid from glucose, with an energy gain in the form of ATP ; the biochemical steps in this pathway are set out in Figure I. Glucose is first phosphorylated by ATP to form glucose-6-phosphate ; this is converted to fructose-6-phosphate, which is then further phosphorylated to form fructose 1,6-diphosphate. Thus two moles of ATP are used in the conversion of one mole of glucose to fructose 1,6-diphosphate. Each mole of fructose 1,6-diphosphate is then split by aldolase into two moles of the triose phosphates, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, which are in equilibrium. The glyceraldehyde-3-phosphate is then converted through a series of intermediates to lactate, two moles of ATP being produced for each mole broken down ; thus a total of four moles of ATP is produced (Figure III).

The known intermediate compounds of this pathway have all been demonstrated in the cell ; Bartlett (1959) gives values for normal red cells. A unique feature of the red cell is its high content of 2,3-diphosphoglycerate (2,3-DPG), which in other tissues is present in only low concentrations. The enzymes catalysing the various steps are discussed in detail by Altmann (1959).

The Pentose Phosphate Pathway.—The pentose phosphate pathway is also known as the

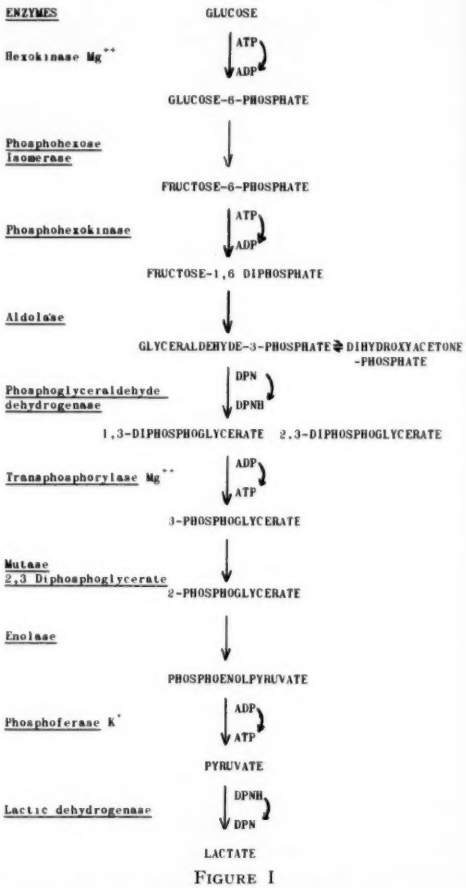

FIGURE I

The Embden-Meyerhof pathway of glucose breakdown

phosphogluconic or hexosemonophosphate pathway. In this pathway glucose is oxidized with the formation of pentose. The first group of reactions may be set out as follows :

$$\text{glucose} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate}$$

$$\text{glucose-6-phosphate} + \text{TPN} \underset{}{\overset{\text{G-6-PD}^1}{\rightleftharpoons}} \text{6-phosphogluconate} + \text{TPNH}$$

$$\text{6-phosphogluconate} + \text{TPN} \underset{}{\overset{\text{6-P-GD}^2}{\rightleftharpoons}} \text{ribulose-5-phosphate} + \text{TPNH} + CO_2$$

[1] G-6-PD = glucose-6-phosphate dehydrogenase.
[2] 6-P-GD = 6-phosphogluconic dehydrogenase.

It will be seen that, for each mole of glucose metabolized by this pathway, two moles of TPN are reduced to TPNH. Some of the ribulose-5-phosphate is then converted through intermediates to glyceraldehyde-3-phosphate, which is then metabolized to lactic acid (Figure II), while some is converted through intermediates to fructose-6-phosphate. The relationship between the pentose phosphate pathway

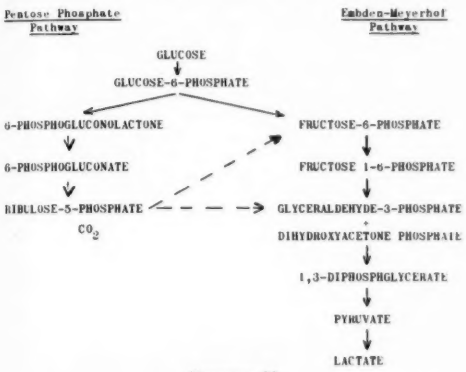

FIGURE II

The relationship of pathways of glucose metabolism in the red cell. The broken arrows indicate the omission of intermediate steps

and the Embden-Meyerhof pathway is summarized in Figure II. A clear detailed diagram of the pentose phosphate shunt is set out by Siperstein (1959), and a full account is given by Dickens (1958).

Energy Production and Utilization.—The energy derived from glycolysis is stored in the red cell mainly in the form of high energy phosphate bonds in ATP. This ATP is produced chiefly via the Embden-Meyerhof pathway, in which the breakdown of one mole of glucose results in a net energy gain of two moles of ATP (Figure III). In addition, some ATP is produced via the pentose phosphate shunt, as the intermediates of this shunt are further metabolized by the Embden-Meyerhof pathway (Figure II). Production of TPNH by the pentose phosphate shunt also contributes to the potential energy of the cell (Murphy, 1960).

The energy produced by glycolysis is used in metabolic processes essential for the maintenance of the structural integrity of the cell. These include maintenance of cation gradients across the cell surface, active transport of material across the cell membrane, turnover of cell components which are renewed throughout the life span, and prevention of autooxidation of hæmoglobin. They are discussed by Prankerd (1955).

Glutathione

The red cell contains relatively large quantities of glutathione, which is present mainly in the reduced form (GSH). It is in a dynamic state, apparently being continually destroyed and resynthesized with a half-life of four days (Dimant *et alii*, 1955). Glutathione is maintained in its reduced state by the action of a specific enzyme glutathione reductase; the coenzyme for this reduction is TPNH. TPNH is generated in the pentose phosphate pathway. The reaction may be set out as follows:

$$\underset{\text{(oxidized glutathione)}}{GSSG} + TPNH + H^+ \xrightarrow[\text{reductase}]{\text{glutathione}} \underset{\text{(reduced glutathione)}}{2GSH} + TPN^+$$

The exact role of glutathione in red-cell metabolism is not known, but evidence suggests that it is in some way related to the maintenance of erythrocyte integrity. Thus there appears to be a relationship between the concentration of reduced glutathione in the cell and the

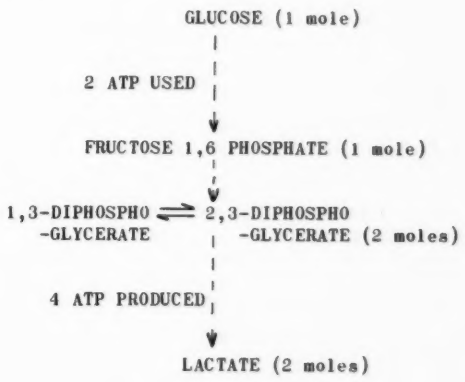

FIGURE III

ATP production from anaerobic glycolysis. The broken arrows indicate the omission of intermediate steps

liability of the cell to hæmolyse during procedures designed to oxidize glutathione (Fegler, 1952); furthermore, reduction of glutathione is defective in subjects with hereditary drug-induced hæmolytic anæmias (see below).

The Aging of the Mature Red Cell

In normal subjects, the average life span of the circulating red cell is 120 days. It is generally postulated that the destruction of

the cell at the end of its life span is due to a failure of one or more critical metabolic activities, as a result of which the cell is unable to produce sufficient energy to maintain its structural integrity; the cell then breaks down and is removed from the circulation by the reticulo-endothelial system. In short, normal red cell destruction has been thought to result from metabolic exhaustion.

This hypothesis has gained support from the indirect evidence that the activities of certain enzymes in the mature cell lessen with age (Allison and Burn, 1955; Marks *et alii*, 1958; Bernstein, 1959). More direct supporting evidence has been the demonstration of a fall in ATP with aging of the cells. Löhr *et alii* (1958) studied enzyme activity and the concentration of glycolytic intermediates in transfused donor cells as they aged *in vivo*. They found that on the sixtieth day after transfusion the intracellular ATP decreased and the ADP correspondingly increased; at this time 70% of the activity of the enzyme phosphoglyceraldehyde dehydrogenase had been lost. They concluded that when the activity of this enzyme is one-third of normal, it is just sufficient to maintain the delivery of the substrate for ATP building; with a further fall in enzyme activity, disturbance of energy production occurs. Bernstein (1959) has also shown that older cells show a decrease in glycolytic rate and in ATP content compared with younger cells. These observations support the view that aging of the cell is accompanied by a decrease in energy production.

Another factor which may contribute to breakdown is progressive loss of lipid or lipoprotein constituents of the membrane during aging. Prankerd (1958) showed that young cells contained more lipid than old cells, and suggested that loss of lipid associated with age might be an important factor in the removal of aged red cells from the circulation, as cells artificially denuded of lipid survived only a short time after transfusion.

Red Cell Metabolism in Hæmolytic Anæmias

Recent studies have demonstrated abnormalities of red-cell metabolism in hereditary spherocytosis, the non-spherocytic congenital hæmolytic anæmias, genetically determined drug-induced hæmolytic anæmia and favism. These abnormalities contribute, at least in part, to the premature destruction of the cells.

Hereditary Spherocytosis

The rate of glucose utilization by mature red cells in hereditary spherocytosis is normal (Selwyn and Dacie, 1954). However, recent evidence has indicated a defect in the intermediary metabolism of glucose in the red cell.

The first indication of a possible abnormality of glycolysis was the observation by Selwyn and Dacie (1954) that the rate of autohæmolysis (that is, the spontaneous hæmolysis occurring in sterile blood incubated at 37° C. for 48 hours), which is significantly increased in hereditary spherocytosis, could be reduced by the addition of glucose to the blood before incubation. Then Prankerd *et alii* (1954, 1955) studied the rate of incorporation and distribution of P^{32} in the phosphates of red cells incubated with P^{32}-labelled orthophosphate, and produced evidence suggesting a basic intracellular defect of phosphorylation. Bertles (1957) has shown that the transport rate of sodium across the erythrocyte surface membrane is abnormal in hereditary spherocytosis, and concludes that, as erythrocyte cation transport is dependent on glycolysis, this observation is consistent with an abnormality in the glycolytic pathway.

It appears that this metabolic abnormality, together with the abnormal shape, results in the premature destruction of the cell. Splenectomy produces a complete clinical remission in hereditary spherocytosis, and red-cell survival after splenectomy is normal or near normal; thus it is obvious that these defects do not significantly reduce life span in the absence of the spleen. How, then, does the presence of the spleen cause hæmolysis?

It is generally considered that, because of its shape, the spherocyte is selectively trapped in the spleen, where it is "conditioned" in such a way that it is incapable of normal survival. Evidence of this conditioning has been the demonstration of an increase in the osmotic and mechanical fragility of cells obtained from the splenic pulp (Emerson *et alii*, 1956). Griggs *et alii* (1960) have shown that this conditioning takes at least ten days to develop; but it is uncertain whether the cells are trapped in the spleen for the full period of the conditioning process, or whether they undergo recirculation with repeated short episodes of stagnation in the spleen.

However, the mere trapping of the cells alone does not appear to account for their destruction, as Harris *et alii* (1957) have shown that normally metabolizing cells which have been made spherical artificially are less susceptible to hæmolysis than the spherocytes of hereditary spherocytosis. Further, Crosby and Conrad (1960) have evidence which suggests that the shape of the cell alone is not responsible for the premature destruction. They studied two subjects with hereditary spherocytosis in

whom an iron-deficiency anæmia with hypochromic and flattened cells was induced by phlebotomy; despite the change in shape of the cells, their short life span was not lengthened, the $T\frac{1}{2}$ of Cr^{51} labelled cells being the same before and after phlebotomy.

It seems probable that the metabolic defect is the major factor predisposing to hæmolysis in the presence of the spleen. Passage through the spleen "stresses" the metabolism of the cell, bringing to light the defect which, in the absence of the spleen, remains clinically latent. The metabolic changes resulting from this stress cause the conditioning of the cell which leads to its premature destruction.

We have studied the intermediates of the glycolytic cycle in hereditary spherocytosis cells. Fresh cells show no striking difference from normal. However, when the blood is "stressed" by incubation *in vitro* at 37° C. for varying periods up to 12 hours, the distribution becomes abnormal—there is a more rapid fall than normal in 2,3-DPG and ATP (although the total nucleotides remain constant), with a more rapid rise in inorganic phosphates (Robinson *et alii*, 1960).

Recent evidence suggests that the glycolytic defect is related to, and possibly causes, a defect in cell lipid. Reed and Swisher (1960) have shown a relationship between erythrocyte energy production, the maintenance of the integrity of the structural lipids of the cell and "membrane function". They state that the hereditary spherocytosis erythrocyte appears to utilize energy inefficiently in maintaining the integrity of its structural lipids. Thus it is possible that the immediate cause of premature destruction is "membrane degeneration" due to loss of cellular lipid.

THE NON-SPHEROCYTIC CONGENITAL HÆMOLYTIC ANÆMIAS

These anæmias form a heterogeneous group of congenital and usually, but not invariably, hereditary hæmolytic anæmias, in which spherocytosis is absent and no abnormal form of hæmoglobin has been demonstrated; they are uncommon, but not excessively rare (Dacie, 1960).

Selwyn and Dacie (1954) found that, on the basis of in-vitro tests, they could be divided into two types (Types I and II). The main difference is in autohæmolysis and the effect of glucose on autohæmolysis. In Type I they found that the autohæmolysis of whole blood alone was normal or only slightly increased, and that the addition of glucose decreased the degree of autohæmolysis. In Type II the autohæmolysis of whole blood alone was greatly increased, and was not decreased by the addition of glucose. Motulsky *et alii* (1955) and Prankerd (1957) each studied several cases (type uncertain) and noted a disturbed intracellular distribution of phosphorylated intermediates.

The most complete results available are those on Type II cases. Selwyn and Dacie (1954) showed that the red cells in this disorder were unable to utilize glucose at a normal rate. De Gruchy *et alii* (1958) studied four Type II cases, and found that, although the increased autohæmolysis was not reduced by glucose, it was reduced by ATP. Analysis of the intermediates in their cases showed a decrease in ATP and a marked increase in total organic phosphate, probably due to an increase in 2,3-DPG. Robinson *et alii* (1960), analysing the intermediates by Bartlett's (1959) method, have confirmed the low ATP and the high 2,3-DPG content of fresh cells; furthermore, they have shown marked differences from normal in the pattern of intermediates on incubation for varying periods up to 12 hours.

Thus there is definite evidence of a defect in glycolysis in Type II cases, and it seems probable that the shortened life span of red cells in this disorder is due to this glycolytic defect, which results in an impairment of energy production and thus in an impairment of structural integrity; this, in turn, results in premature destruction. The exact nature of the glycolytic defect is yet to be established.

It has been pointed out above that the non-spherocytic congenital hæmolytic anæmias are a heterogeneous group. Review of reported cases suggests that Type I represents several different sub-types which, although similar in autohæmolysis, differ in red-cell morphology and osmotic fragility; in Type II the morphology and fragility are more constant. It seems likely, not only that Type I and Type II differ in their biochemical defect (Robinson *et alii*, 1960), but that the intragroup variations in Type I are due to different biochemical defects. Thus in some cases an enzyme defect similar to that in "'Primaquine'-sensitive hæmolytic anæmia" has been described (Newton and Bass, 1958; Shahidi and Diamond, 1959; Zinkham and Leonard, 1959). In the cases of both Type I and Type II studied by the present writer, the G-6-PD activity has been normal (de Gruchy *et alii*, 1960). It is obvious that further metabolic studies correlated with the clinical and hæmatological findings are required to determine the number of different types of biochemical defect in these anæmias, and whether a particular biochemical defect results

in a particular pattern of change in red-cell morphology, osmotic fragility and auto-hæmolysis.

DRUG-INDUCED HÆMOLYTIC ANÆMIAS IN SENSITIVE INDIVIDUALS

It is well known that the administration of certain drugs, which cause no ill effects on the blood of most persons, will occasionally produce a hæmolytic reaction in sensitive individuals. The nature of the sensitivity responsible for the hæmolysis has been poorly understood. In only a few cases have antibodies active against red cells in the presence of the drug been demonstrated, and it would seem that the majority of cases of drug-sensitive hæmolytic anæmia are not due to an immune mechanism.

In 1952, Hockwald *et alii* noted that between 5% and 10% of otherwise healthy American Negro troops developed an acute hæmolytic anæmia after the administration of " Primaquine ". Before exposure to the drug there was no evidence of hæmolysis in these sensitive Negroes, and their red cells were morphologically normal and of normal osmotic and mechanical fragility. These observations resulted in an intensive and fascinating study of the red cells of sensitive individuals, initiated by Alving and his collaborators in the Army Malaria Research Unit at the Stateville Penitentiary, Illinois, as a result of which it has been shown that the red cells have a genetically determined enzyme defect. Thus the drug sensitivity is due to an inborn error of metabolism.

It seems probable that many cases of acute hæmolysis caused by drug sensitivity are due to the presence of the same red cell enzyme defect as has been demonstrated in " Primaquine " sensitivity. The elucidation of this defect has resulted, not only in an explanation of the mechanism of many previously unexplained drug-induced hæmolytic anæmias and the condition of favism, but also in a significant increase in our knowledge of red-cell metabolism.

Nature of the Red-Cell Defect

The first metabolic abnormality demonstrated in the red cells of " Primaquine "-sensitive subjects was a deficiency of reduced glutathione (Beutler *et alii*, 1955). This led to an investigation of the metabolic reactions maintaining glutathione in its reduced form in the cell (see pentose phosphate pathway and glutathione) resulting in the demonstration of a deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) (Carson *et alii*, 1956). Deficiency of this enzyme results in a decreased generation of TPNH; TPNH is necessary for the conversion of oxidized glutathione to reduced glutathione, and thus its deficiency results in a deficiency of reduced glutathione. The deficiency of G-6-PD is now considered to be the basic enzymatic defect in the sensitive cells. However, although G-6-PD is quantitatively deficient in sensitive cells, in-vitro studies have not revealed any qualitative difference between the enzyme in sensitive and normal cells (Kirkman, 1959). Recently Rimon *et alii* (1960) have reported that normal red-cell stromata contain an activating factor for the G-6-PD of sensitive cells; this activating factor is absent from the stromata of sensitive cells. It is possible that lack of this factor in " Primaquine "-sensitive cells represents the primary expression of the genetic defect.

A number of other biochemical abnormalities have been demonstrated in sensitive cells. Thus there is an increased activity of glutathione reductase and aldolase and decreased activity of catalase; TPN and DPN are increased, but TPNH and DPNH are reduced, and the total levels of these coenzymes are normal (Alving *et alii*, 1958; Tarlov and Kellermeyer, 1959). These abnormalities are considered to be either secondary or compensatory to the G-6-PD deficiency.

Although it is generally accepted that the metabolic defect is responsible for hæmolysis of the cell when it is challenged by " Primaquine " and other drugs, the exact mechanism by which it causes the hæmolysis is not yet known. Jandl and Allen (1960) have shown that the aromatic amines which produce hæmolysis cause oxidative denaturation of hæmoglobin and its precipitation as Heinz bodies; exposure of red cells alone to oxygen *in vitro* slowly produced the same sequence of oxidative injury as did drugs. They suggest that the hæmolytic anæmia with Heinz body formation due to the drug hypersensitivity represents an acceleration of normal mechanisms of red cell aging.

Racial and Genetic Features

The defect occurs predominantly in Negroes; nevertheless, it also occurs in the white (Caucasian) population. In non-Negro races the highest incidence is in Italians, particularly Sardinians, in some Greeks and in Sephardic (dark-complexioned Oriental) Jews; it is rare among Ashkenazic Jews (light-complexioned Jews from eastern, central and western Europe). Marks and Gross (1959*a*), in an analysis of 666 subjects in San Francisco and New York, found an incidence of 13·0% in the Negro and of 0·7% in the Caucasian population.

Genetic studies suggest that the defect is probably transmitted by an incompletely dominant sex-linked (*x*-chromosome) gene with

variable expressivity. Males are affected more commonly than females, and in general females are less severely affected. Most affected males show full expression; thus full expression occurs in about 15% of American Negro males and in 2% of Negro females. On the other hand, most females and occasional males show intermediate expressivity; probably about 5% of American female Negroes are intermediates. Genetic features are discussed by Childs *et alii* (1958) and by Marks and Gross (1959*a*). Recently Marks and Gross (1959*b*) have produced evidence of a quantitative difference in the erythrocyte G-6-PD deficiency between Negroes and Caucasians.

Clinical Features

Self-Limiting Nature of the Hæmolytic Episode.—The severity of the hæmolytic episode induced by a particular drug is related to its dose (see below). The most detailed study of the clinical course has been in " Primaquine "-sensitive volunteers, who received a dose of 30 mg. of " Primaquine " per day. This resulted in a self-limited hæmolysis, beginning in three to four days and lasting for about seven days, and followed by a return of the hæmoglobin value to normal on the twentieth to thirtieth day, despite continued administration (Dern *et alii*, 1954). The self-limiting nature of the hæmolysis is due to the fact that cell sensitivity is a function of cell age; older cells are destroyed, while younger cells are resistant (Beutler *et alii*, 1954). Hæmolysis ceases when older populations of cells have been destroyed and only the younger ones remain. However, the resistance of younger cells is relative, as a second hæmolysis can be induced if the dose is suddenly greatly increased (Kellermeyer *et alii*, 1959).

Drugs which may Cause Hæmolysis: Relation to Dosage.—Most of the experimental work elucidating the nature of the red-cell defect has been done by studying the effect of " Primaquine " on sensitive individuals, and for this reason the condition is sometimes called " ' Primaquine '-sensitive hæmolytic anæmia ". However, it is now realized that " Primaquine " is simply one member of a large number of compounds which may cause a hæmolytic reaction in sensitive individuals.

The drugs shown to cause hæmolysis are set out in Table I (a number are commonly used drugs).

It seems probable that this list will be considerably added to as more drugs are tested on sensitive subjects.

There is a remarkably constant relationship between the dose of a particular drug and the degree of hæmolysis it produces (Kellermeyer *et alii*, 1959). Thus aspirin, when given in small doses, causes little if any hæmolysis; however, when used in doses commonly recommended for the treatment of rheumatic diseases, it causes definite hæmolysis. Kellermeyer *et alii* (1958) found that aspirin (8 grammes), " Lederkyn " (4 grammes), " Furadantin " (600 mg.) and " Furoxone " (400 mg.) produced moderate intravascular hæmolysis when given in the daily dosages mentioned to " sensitive " individuals with normal kidney function. Hæmolysis due to vitamin K derivatives and naphthalene (moth balls) is discussed by Zinkham and Childs (1957).

Impairment of Renal and Liver Function as a Factor Predisposing to Hæmolysis.—It is obvious that in persons with an impaired mechanism of drug elimination, smaller doses will cause hæmolysis, and that the doses listed above would cause a more severe hæmolysis than usual.

TABLE I

Aspirin	" Furadantin " (nitrofurantoin)
Phenacetin	" Gantrisan " (sulphafurazole)
Sulphonamide	" Lederkyn ", " Kynex " (sulphamethoxypyridazine)
Sulphapyridine	" Benemid " (probenecid)
" Primaquine "	" Furoxone " (furazolidone)
" Pamaquine "	" Salazopyrin " (azulfidine)
Naphthalene	" Solutox ", " Albucid " (sulphacetamide)
Dimercaprol (BAL)	" Diasone " (sulfoxone)
Certain vitamin K derivatives	

This point is of particular importance in relation to the drugs used in treating urinary infection—namely, " Furadantin ", " Lederkyn " and " Gantrisan " — as the underlying disorder causing the infection may also cause some degree of impairment of renal function. Thus " Furadantin " has caused hæmolysis in a daily dosage of 400 mg. (Kimbro *et alii*, 1957). Severe liver disease, by impairing metabolic breakdown of drugs, may increase plasma concentration and thus predispose to hæmolysis.

Infection.—Infection may act as a predisposing or precipitating factor. Szeinberg *et alii* (1958) found that hæmolysis occurred in sensitive subjects after infectious disease (typhoid, viral infections) and " Asian 'flu ".

Hæmatological Changes. — Hæmatological changes during hæmolysis are described by Beutler *et alii* (1954). The changes are those of acute hæmolytic anæmia with Heinz body formation; the result of the Coombs test is negative. Recent observations suggest that sensitive red cells have a somewhat shorter life span than non-sensitive cells. Thus drug-sensitive individuals, although asymptomatic,

can technically be considered to have " chronic hæmolysis without anæmia " even prior to the ingestion of hæmolytic agents (Alving, 1960).

Laboratory Detection of Sensitive Individuals

A number of tests have been used to detect sensitive individuals. They are the Heinz body test (Beutler *et alii*, 1955), the glutathione (GSH) stability test (Beutler, 1957, 1959), assay of glucose-6-phosphate dehydrogenase (Marks, 1958), the cresyl-blue dye test (Motulsky and Campbell, 1960) and the methæmoglobin reduction test (Brewer *et alii*, 1960).

Method of Choice.—In any person definitely suspected of having the disorder, assay of G-6-PD should be performed to establish the diagnosis conclusively. However, the assay requires special laboratory facilities, and in general is not suitable as a routine diagnostic laboratory procedure. As a screening test both in clinical diagnosis and in population studies, the two most widely used tests have been the GSH stability test and the cresyl-blue dye test. However, it now appears that the relatively simple methæmoglobin reduction test is the method of choice as a screening test. In particular it has proved more sensitive than other screening tests in the detection of females with an intermediate susceptibility to hæmolysis (Brewer *et alii*, 1960).

FAVISM

Favism is an acute hæmolytic anæmia resulting from the ingestion of beans or the inhalation of the pollen of the plant, *Vicia fava*, by susceptible inhabitants of Mediterranean countries (Luisada, 1941). Recently it has been shown that persons susceptible to favism have the same enzymatic red-cell defect (G-6-PD deficiency) and the same pattern of inheritance as persons with " Primaquine " sensitivity (Sansone and Segni, 1957; Szeinberg *et alii*, 1958; Zinkham *et alii*, 1958). Thus acute hæmolysis occurs when " Primaquine " and other hæmolytic drugs are administered to a person with favism (Larizza *et alii*, 1958).

However, it appears probable that some other factor or factors are involved in the hæmolytic attack which follows exposure to fava beans. Thus, Szeinberg *et alii* (1958) found that several persons known to have sensitive red cells had eaten fava beans without hæmolysis occurring. Furthermore, hæmolysis from fava beans may be extremely variable at various periods of an affected subject's life, and the first attack may not occur until late in life (Alving *et alii*, 1960).

Favism in Australia

Recently favism has been reported in Australia (Brooks *et alii*, 1958; Harley and Dods, 1959), and the writer knows of several unreported cases in which the patients' families have been studied and shown to have sensitive cells. Harley and Dods (1959) have pointed out that the common broad beans grown and eaten in Australia are in fact fava beans, and that, with the increase of migrants, about 2·4% of the Australian population are of Mediterranean origin.

SUMMARY

1. The red cell is in a state of continual metabolic activity and can be regarded as a living cell, in the sense that it exists in a dynamic state, does work and requires sustenance to maintain its energy metabolism.

2. The cell derives energy from the breakdown of glucose via the Embden-Meyerhof and pentose phosphate pathways.

3. The structural and functional integrity of the cell is largely dependent on this energy production.

4. Destruction of the cell at the end of its life span *in vivo*, and the loss of viability which occurs when blood is stored *in vitro* for transfusion, are due to a failure of energy production as a result of the running down of metabolic activity.

5. The congenital hæmolytic disorders, hereditary spherocytosis and the non-spherocytic congenital hæmolytic anæmias are associated with abnormalities of red-cell metabolism; the premature destruction of red cells in these disorders is due, at least in part, to these metabolic defects.

6. Many cases of acute drug-induced hæmolytic anæmia and the condition of favism are due to a genetically determined red-cell enzyme defect—namely, glucose-6-phosphate dehydrogenase deficiency. These anæmias are assuming increasing clinical importance in Australia with the recent increase in number of persons of Mediterranean origin, in whom there is a relatively high incidence of this metabolic defect.

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Proceedings of The Royal Australasian College of Physicians

ANNUAL MEETING, 1960

The Annual Meeting of the College in 1960 was held in Melbourne from May 25 to 28. It was attended by over 200 Fellows and Members representative of all the Australian States and New Zealand. The President, Dr. T. M. Greenaway, was in the chair. Professor John McMichael, M.D., F.R.C.P., F.R.S., Sir Arthur Sims Commonwealth Travelling Professor for 1960, attended the meeting and contributed to discussion at the scientific and clinical sessions.

College Ceremony

The Annual Ceremony of the College was held in the Wilson Hall, University of Melbourne, on Wednesday, May 25, in the presence of His Excellency the Governor of Victoria and Lady Brooks. An audience of 800 was present.

Addresses were given by the President and His Excellency the Governor, and messages of greeting from the Royal College of Physicians of London were delivered by Dr. Wallace Brigden, and from The American College of Physicians by Dr. George C. Griffith. Newly-admitted Fellows and Members were then presented to the President. Dr. J. A. L. Matheson, M.B.E., Ph.D. (Birmingham), M.Sc., Vice-Chancellor of Monash University, then delivered the Arthur E. Mills Memorial Oration entitled "Engineering and Medicine". At the conclusion of the ceremony guests were entertained at supper in the University Union.

College Dinner

The College Dinner was held at the Hotel Australia, Collins Street, Melbourne, on the evening of May 26. The guests of honour were the Sir Arthur Sims Commonwealth Travelling Professor, Professor John McMichael, Dr. George C. Griffith, official representative of The American College of Physicians, Dr. Wallace Brigden, the R. T. Hall Overseas Lecturer of The Cardiac Society of Australia and New Zealand for 1960, Mr. Julian Orm Smith, representing the President of the Royal Australasian College of Surgeons, Dr. Ewen Downie, President of the Endocrine Society of Australia, Professor Bruce Mayes, Chairman of the Australian Regional Council of the Royal College of Obstetricians and Gynæcologists, and Dr. T. E. Lowe, Chairman of the Second Asian-Pacific Congress of Cardiology. The toast of the College was proposed by Professor J. G. Hayden and acknowledged by the President, Dr. T. M. Greenaway, who then proposed the toast of the guests. Professor McMichael and Dr. George C. Griffith spoke in reply.

Plenary and Scientific Sessions

Plenary Session

A plenary session with the Endocrine Society of Australia was held in the Anatomy Lecture Theatre of the University of Melbourne on Wednesday, May 25.

Dr. Maurice L. Wellby presented a paper prepared in conjunction with Dr. Basil S. Hetzel, entitled "Paper Chromatographic Studies of the Circulating Thyroid Hormone in Man". They reported that recently introduced techniques of paper chromatography had been of great value in the further elucidation of the nature of the circulating thyroid hormone, the precise nature of which remained uncertain. Metabolic studies from their laboratory suggested the possibility that under thyrotropic hormone (TSH) stimulation, the thyroid produced a rapidly acting hormone which was distinct from the thyroid hormones already known. This led to a reexamination with paper chromatographic techniques of the nature of the thyroid secretion under conditions of thyroid activation in both physiological and pathological states. The method was developed from existing methods, and both radio-active and chemical methods of detection of the iodine-containing components were used. Thyroxine (T_4) was found to be the only circulating thyroid component in normal subjects under conditions of basal secretion, but after TSH administration, traces of triiodothyronine (T_3) occurred, as well as increased amounts of T_4 in proportion to the increase in P.B.I. In hyperthyroidism T_4 was found in all cases; T_3 was found in nine cases out of 10 by radio-active methods, and in six cases out of 15 in untreated cases by the chemical method. Iodotyrosines (I.T.) were detected in seven cases out of 10 with radio-active methods, and in five cases out of 15 with the chemical method. I.T. was detected also in plasma from a goitrous cretin. Radio-iodine was known to damage the thyroid gland, and the detection of T_3 and I.T. after its use was considered to be due to that damage. However, in this study the chemical detection of T_3 in thyrotoxicosis indicated that it was not an artefact. In addition, the finding of I.T. in thyrotoxic plasma before any therapy showed that this substance could be produced by the gland where radiation had not occurred. The occurrence of I.T. was of great interest, because it pointed to the possibility of a disturbance in biosynthesis of the thyroid hormone in those cases of thyrotoxicosis, such as had been demonstrated in cases of goitrous cretinism. However, so far the correlation of the detection of I.T. in thyrotoxicosis with any other clinico-pathological features, such as age, size and histology of the thyroid, tanned red-cell titre, or the level of plasma P.B.I., had not been accomplished.

A paper was presented by Ian Hales, entitled "Suppression of Thyroid Function—Its Diagnostic, Physiological and Therapeutic Significance". Under the heading of diagnosis Dr. Hales said that the

activity of the thyroid could not be reduced by exogenous thyroid in the presence of thyrotoxicosis, as that formed a basis for the assessment of elevated I^{131} uptakes. The radioactive iodine uptake rate was studied before and after the administration of thyroxine (0·4 mg. per day for three weeks) to 344 euthyroid patients and 105 thyrotoxic patients. There was a mean rise in uptake in thyrotoxic patients, while in the euthyroid patients there was a fall in uptake rate. Triiodothyronine (120–150 μg. per day) was given for seven days to 85 euthyroid and 41 thyrotoxic patients. Again there was a mean rise of uptake in thyrotoxic subjects and a fall in euthyroid subjects. In most cases, either test gave a clear-cut answer. The number of doubtful results might have been slightly higher with triiodothyronine, but the result was obtained sooner, and appeared not to be affected by prior medication. The use of "Neomercazole" and a thyroxine suppression was useful in further assessing doubtful cases. Discussing physiology, Dr. Hales then reported that the increase in iodine uptake rate during suppression could be due to an altered sensitivity to hormone, or to an increased blood flow. It had been suggested that failure of suppression was a feature of Graves's disease and not of the state of hyperthyroidism; but in 36 cases studied at various times after I^{131} therapy, suppression had been shown as early as 12 weeks after therapy. Suppression had been demonstrated as early as 12 weeks after "Neomercazole" therapy. Three euthyroid patients with active exophthalmos demonstrated suppression. It was suggested that the loss of suppression was related to the hyperthyroidism, and not to the underlying Graves's disease. Finally, under the heading of therapy, Dr. Hales said that the use of thyroid suppression to reduce the size of goitres was a well-established therapeutic procedure. It was also of diagnostic assistance in the following ways: (i) In children, reduction of the size of the goitre might exclude thyrotoxicosis and carcinoma. If the size was not reduced, an I^{131} uptake study could be performed during the thyroid medication, in which case the gland would be protected from radiation except in thyrotoxicosis. (ii) Reduction in the size of the gland might clarify the differentiation between multinodular and single-nodule goitre. The use of thyroid suppression was also useful in the management of carcinoma of the thyroid, in the treatment of exophthalmos, and in the treatment of thyroiditis.

Dr. J. Bornstein then presented a paper entitled "A Directly Acting Insulin Antagonist of the Pituitary and its Possible Clinical Significance". He said that, following on the observation of Baird and Bornstein, that after acid alcohol extraction the residue of plasma of both normal and diabetic patients contained a factor capable of inhibiting glucose utilization by rat diaphragm, it was shown by Bornstein and Hyde that that factor was present at a higher concentration in diabetic patients with arterial complications than in those who had a similar history but without complications. The finding that that material was ultrafiltrable and not present in the plasma of hypophysectomized patients led to the investigation of the human pituitary as a source of the "hormone". The glands were collected at autopsy and stored in acetone. Prior to extraction they were dried, and then extracted with an acid-solvent mixture until only trivial amounts of protein were found in the extract. The residue was incubated for four hours at 37° C. and then dried *in vacuo*. The dry cake was pulverized and extracted with 0·1M acetic acid. The acid extract was ultrafiltered through "Cellophane" and the ultrafiltrate chromatographed on "Zeocarb 225H". The middle peak obtained was freeze-dried and then tested. It was shown to inhibit glucose use by muscle, glucose oxidation by liver, protein synthesis by muscle and fatty-acid synthesis by liver, but to accelerate cholesterol synthesis by the liver. On its injection into rabbits, it was found that within 24 hours the blood cholesterol and blood ketone levels were elevated, and within 48 hours the blood amino acid levels. No effect on the rabbit's blood sugar level was observed within 96 hours. The material was electrophoretically substantially homogeneous, and on centrifugation in the artificial boundary cell gave a M.W. of 4400, which corresponded with the amino-acid analysis. The possible clinical significance and proposed course of investigation were discussed.

Dr. J. M. Greenaway then read a paper entitled "Protein Binding of Hydrocortisone in Health, Pregnancy and Some Disease States". He said that the early work on the binding of cortisol by plasma proteins suggested that about half was bound to plasma globulins. Those results were invalid owing to the low sensitivity of the existing analytical techniques; the introduction of 4-^{14}C-cortisol had shown that about 95% of plasma cortisol was protein-bound at physiological steroid concentrations. The concentration of transcortin could be assessed by adding 4-^{14}C-cortisol to a sample of plasma dialysed against albumin in saline. Any retention of the steroid by the plasma was regarded as due to transcortin; the decrease in binding caused by increasing cortisol concentrations indicated the concentration of transcortin. Transcortin concentrations and total cortisol concentrations were estimated, and the freely diffusible cortisol concentration was calculated, in plasma from healthy controls, from women in the last trimester of pregnancy and patients suffering from sarcoidosis and myelomatosis. In pregnancy the concentration of transcortin was significantly elevated in comparison with the controls, as was the total plasma cortisol concentration. However, the freely diffusible cortisol concentration was identical with that of the controls. In the patients suffering from sarcoidosis, the total and freely diffusible cortisol concentration and the concentration of transcortin were virtually the same as those of the controls. Those findings applied irrespective of the clinical condition of the patient and the electrophoretic pattern of plasma proteins. In myelomatosis there was a tendency towards low concentration of transcortin and high concentration of freely diffusible cortisol relative to the total plasma cortisol concentrations. The significance of these findings was discussed, and it was concluded that a knowledge of the total plasma cortisol concentration did not necessarily give a true indication of the steroid concentration confronting the tissues, or an indication of the rate of cortisol secretion by the adrenal cortex.

A. W. Steinbeck presented a paper on "Cushing's Syndrome and its Diagnosis with the Help of Steroid Studies", and illustrated it with slides of graphs and tables and "Kodachromes". He said that the diagnosis of Cushing's syndrome was considered to be often disregarded, but if established, necessitated radical treatment. Accordingly the diagnosis must be certain but, because of the poor prognosis of the untreated disease, also early. That was difficult in those cases in which the features were not predominantly classical. A review of the clinical features of some 23 cases of Cushing's syndrome confirmed this, and in addition, suggested reasons for delay in diagnosis. The plasma levels of free steroidal dihydroxyacetones (SDHA), before and after an infusion of corticotropin, were studied in some of those patients. Before corticotropin had been given, the levels could be normal or elevated, sometimes variable, and in patients with adrenal hyperplasia they tended to rise pro-

gressively over the time of infusion, and to higher levels than in normal subjects. One patient with an adrenal adenoma—the only functional adrenal tissue—showed a similar response. Urinary steroids were studied as 24-hour excretions of 17-ketosteroids (17-KS), 17-ketogenic steroids (17-KGS), 17-hydroxycorticosteroids (17-(OH)CS), steroidal dihydroxyacetones (SDHA) and 21-deoxyketols (DOK), before and after infusion of corticotropin. Resting excretions were either normal or raised, and after corticotropin, on the day of its administration, the excretions rose to either a normal or a greater than normal extent. In that regard, an increased duration of excretion seemed most characteristic in adrenal hyperplasia, although it also occurred in the case of the adrenal adenoma, and proportionately, changes were often greatest for 17-(OH)CS and SDHA groups and least in the 17-KS group. However, other cases existed in which the clinical and steroidal features suggested a Cushing's process, but not decisively. Nevertheless, the steroidal changes seemed to have practical diagnostic value.

Scientific Sessions

The first scientific session was held in the Anatomy Lecture Theatre on May 26.

Wallace Brigden, in a paper entitled "Nutritional Heart Disease", reported that the clinical features, natural history and pathology of myocardial disease due to excessive consumption of alcohol had been studied in a series of 36 patients. Only two were women. All had consumed vast quantities of alcohol for 10 to 25 years, and their ages ranged from 35 to 60. Every patient had unequivocal evidence of myocardial disease and no symptoms or signs of coronary occlusion, valve disease or hypertension. The onset was usually insidious, but two patients presented with congestive failure of the high-output type characteristic of beriberi and responding to thiamine; in one case that had occurred 20 years before death in chronic hypokinetic failure, which had responded to the usual measures but not to thiamine. Arrhythmias occurred at some time in at least one-third of cases, and atrial fibrillation was usually intermittent for years before becoming permanent. The electrocardiograph showed a variety of abnormalities, the severity of which bore some relation to the stage of the disease. Thus normal conduction with minor *T*-wave changes was usual in the early phases, whilst some degree of intraventricular or extraventricular block was common in the later phases. Chronic irreversible hypokinetic failure was characteristic of the later stages of the disease. Six necropsies showed no significant coronary disease and no macroscopic fibrosis. The left ventricle was enlarged, and on microscopic examination there were scattered small areas of muscle necrosis and/or fibrosis. Cell reaction was not conspicuous. When a lesion was adjacent to the endocardium, a small organized mural thrombus was often present. It was noted that in that series hepatic cirrhosis and peripheral neuropathy were rare and slight.

In a paper entitled "Constrictive Pericarditis", M. J. Etheridge described the clinical features and results of operation in a group of 14 patients with constrictive pericarditis admitted to the Royal Melbourne Hospital since 1948. The patients studied were aged from 17 to 59 years. There were nine males and five females. Two patients presented an acute course with the signs of constriction developing during recovery from acute tuberculous pericarditis. The diagnosis had been confirmed at operation in 12 cases. Limitation of effort tolerance, a small pulse, raised jugular venous pressure, œdema and liver enlargement were the commonest clinical features; pulsus paradoxus, ascites and splenomegaly were less frequent. A third heart sound was usually audible, and the heart shadow was small or only moderately enlarged. Pericardial calcification had been present in half of the patients. The characteristic electrocardiographic features of constrictive pericarditis were described, and a step-like *P* wave similar to the dominant left-atrial *P* wave of mitral stenosis had been noted in four instances. Pericardial resection was followed by a good result in eight patients, including the two with constriction following tuberculous pericarditis. There had been no deaths from operation. In four patients an unsatisfactory result followed pericardectomy. Relevant factors were cardiac enlargement with atrial fibrillation, cirrhosis of the liver, coincidental mitral valve disease and technical difficulties in resecting the pericardium. The ætiology of constrictive pericarditis was briefly discussed, and the difficulty in differentiation of that disease from some cases of diffuse myocardial fibrosis was emphasized.

A paper entitled "Hereditary Spherocytosis and Gout in a Single Large Family", by J. R. Lawrence and H. N. Robson, was presented by the latter. It was stated that the study had been undertaken in an attempt to obtain information on the pattern of transmission of hereditary spherocytosis and of gout, as controversy continued on both topics, and also to discover if there was any evidence of genetical linkage between the two disorders. The family consisted of 169 members, 134 of whom were examined clinically and hæmatologically; the hæmatological tests included immediate and delayed osmotic fragility and auto-hæmolysis tests and blood uric acid determinations. The transmission of hereditary spherocytosis in that family was found to conform exactly to a single autosomal dominant mechanism. There had been no evidence of genetical linkage between the two conditions.

In a paper entitled "Non-Specific Factors in Immunity against the Action of Bacterial Exotoxins in the Experimental Animal", by E. A. North and Hazel M. Doery, E. A. North presented the results of experimental work showing that the enzyme phosphatidase A, a constituent of venoms of many snakes and insects, injected into animals, gave protection against subsequent challenge by staphylococcal, diphtheria and tetanus toxins. It was recalled that phosphatidase A split off a long-chain fatty acid from lecithin, present in all animal cells, leaving lysolecithin. When the effects of both products of the action of that enzyme were considered, it was found that only the ionizable salts of unsaturated fatty acids neutralized exotoxin *in vitro*, whilst lysolecithin protected mice when injected intravenously separately from toxin; but its mode of action was uncertain. However, it had been shown that ganglioside, a compound containing neuraminic acid, prepared from ox brain, neutralized toxin. Neither fatty acid or ganglioside was effective in the presence of serum protein. There was evidence to support the idea that ganglioside normally present in an insoluble form was made soluble by lysolecithin as a result of its high surface activity. Phosphatidase A was known to be present in the pancreas, and it was suggested that the liberation of bacterial toxin in the body might bring a natural genetically-controlled defence mechanism into action. The concept visualized involved the release of fatty acid and lysolecithin by phosphatidase A. If gangliosides were then made soluble by lysolecithin, both fatty acids and gangliosides would be available for adsorption of toxin.

M. R. Playoust, in a paper entitled "Metabolic Balance Studies in a Patient with Wilson's Disease", said that a male, now aged 19 years, had first developed incoordination and tremor eight years earlier. The diagnosis of Wilson's disease was made in 1956, and since then he had been treated with intermittent courses of B.A.L. Recently, more extensive studies of urinary copper excretion had been carried out to compare the effects of penicillamine, B.A.L. and a combination of both agents. Full copper balance studies had confirmed that the greatly increased urinary excretion during penicillamine therapy was due to a mobilization of the body's copper stores, and that absorption from the gastro-intestinal tract was not altered significantly. An unusual feature in that patient had been the unexpected finding in 1959 of an increased urinary calcium output, although he had been normocalciuric in 1956. Calcium metabolic studies showed that he was in negative balance; but that was corrected, with decrease in the urine calcium excretion, when he was given cortisone. The implications of the studies were discussed; apart from slightly increased amino-aciduria, there had been no biochemical or histological evidence of renal or hepatic dysfunction. It was suggested that the skeleton was the primary site of his disorder of calcium metabolism.

The second scientific session was held in the afternoon of May 27, in the Anatomy Lecture Theatre.

V. J. Kinsella, W. B. Hennessy and E. P. George, in a paper presented by W. B. Hennessy, discussed "Studies on Post-Gastrectomy Malabsorption: The Importance of Bacterial Contamination of the Upper Small Intestine". It was noted that severe nutritional problems after gastrectomy were uncommon; but on the other hand, weight loss of varying degrees was encountered in at least half the patients. The two main reasons for that were reduced food intake and malabsorption. In order to determine the frequency of malabsorption after gastrectomy, fat absorption studies using I^{131}-glyceryl trioleate were carried out on 28 patients, and in 18 of them steatorrhœa had been demonstrated. The factors responsible for post-gastrectomy malabsorption were multiple, and included (i) improper admixture of food with bile and pancreatic secretion, (ii) increased intestinal transit time, and (iii) changes in the small-bowel mucosa itself. However, the authors found that bacterial contamination of the upper part of the small intestine was an important additional cause of post-gastrectomy steatorrhœa and weight loss, and advised that that mechanism be suspected when an obstructed duodeno-jejunal loop was demonstrated. Ten patients with afferent loop obstruction after the Billroth II operation had been studied, and it was suggested that they presented examples of the blind-loop syndrome. Two patients illustrating that mechanism were presented. Each had an obstructed afferent loop from which a variety of bacteria were cultured at laparotomy. Each responded favourably to antibiotics, and the malabsorption in one disappeared after duodeno-jejunostomy. However, nutritional problems following gastrectomy might be complex and varied, and two unusual cases in illustration were described. In one of them volvulus of the whole of the small intestine occurred, and a remarkable improvement followed treatment with sulphaguanidine, while the other was associated with multiple pancreatic non-insulin-secreting microadenomata.

A paper on "The Absorption of Folic Acid", by W. R. Pitney and R. A. Joske, was presented by W. R. Pitney. A folic-acid absorption test was described, in which serum concentrations of folic acid were measured for three hours after an oral dose of 2 mg. of folic acid as sodium folate. Measurements were performed by a microbiological technique, using a folic-acid-sensitive mutant strain of *Streptococcus lactis*. In normal individuals, peak serum concentrations were usually found at the end of the first hour and ranged from 70 to 130 μμg. per millilitre. In tests on patients, peak values below 60 μμg. per millilitre were taken to indicate malabsorption of folic acid. The folic-acid absorption test was carried out on 26 patients with clinical and laboratory evidence of the intestinal malabsorption syndrome. The result was abnormal in 14 (54%). There was no significant correlation between the results of the folic-acid absorption test and those of other absorption tests. In 23 patients tested by both the folic-acid and the xylose absorption tests, at least one result in 19 was abnormal. It was concluded that the folic-acid absorption test might be useful in the diagnosis of intestinal malabsorption. Twenty-seven patients with megaloblastic anæmia were tested. The serum vitamin B_{12} concentration (Euglena assay) was less than 100 μμg. per millilitre in 17 patients, and these were considered to have megaloblastic anæmia due to vitamin B_{12} deficiency. Thirteen patients suffered from pernicious anæmia, and all absorbed folic acid normally. Four patients had vitamin B_{12} deficiency due to intestinal malabsorption, and two of them showed low folic acid absorption. Ten patients showed vitamin B_{12} concentration greater than 100 μμg. per millilitre. In these the anæmia was considered due to folic acid deficiency, and all responded clinically to folic-acid therapy. No patient had clinical evidence of intestinal malabsorption. Two were aboriginal women in whom anæmia was first noticed during lactation. Both showed very low folic-acid absorption curves as well as other laboratory evidence of malabsorption. In one patient anæmia developed while she was taking anticonvulsant drugs; that patient also showed an abnormal folic-acid absorption curve. Seven patients were considered originally to have nutritional megaloblastic anæmia. Three of the four who absorbed folic acid normally showed other laboratory evidence of intestinal malabsorption. It was concluded that the folic-acid absorption test was useful in the study of the mechanism of megaloblastic anæmia. Nutritional megaloblastic anæmia should be diagnosed only after intestinal malabsorption had been excluded.

G. V. Davies, in a paper entitled "The Relation of Physical and Mental Disorders in Older Women", said that the fact that 12% of the Australian population aged over 60 years supplied 30% of the first admissions to mental hospitals was leading to renewed interest in the mental health of the elderly. In 1959 a survey of the subject had been attempted in the Mental Hygiene Department of Victoria. A previous pilot study had shown that there was more brain damage in men of that age group by the time they reached mental hospitals; therefore the study was confined to women. Three groups of 50 women were included: one group was from admissions to a mental hospital, one from a geriatric unit, and one from an elderly persons' club. It was found that there was considerable physical ill-health in the mental hospital patients and considerable psychiatric disability in the patients in the geriatric unit, and that the sample group from the elderly people's club showed, on the average, a departure from normal physical and mental health. The mechanisms of the relationship between physical and mental disorders in the elderly were summarized in the following manner. (i) Sociological: physical ill-health of one person was shown as a cause of mental disturbance in another. (ii) Genetic: in senile dementia, the parents of the patients showed 20% more senile dementia than was in the community. (iii) Cerebral degeneration: brain

damage from any cause might lead to psychosis; but that was partly dependent on the personality of the patient. (iv) Disease outside the brain: that included congestive heart failure, anæmia and metabolic diseases. (v) Psychosomatic influences: some of the signs and symptoms of old age itself were psychogenic. (vi) Somatopsychological factors: physical illness might lead to depression and other affective disorders.

In a paper by H. J. H. Colebatch and D. F. J. Halmagyi, presented by H. J. H. Colebatch, and entitled "Lung Mechanics in Experimental Non-Fatal Drowning", they recalled that in a previous study (Proceedings of the College, February, 1960), they had shown that severe hypoxia—sufficient to threaten survival—might follow the introduction of small quantities of sea or fresh water into the lungs. Further understanding of the fluid-induced changes had been obtained in the present study of lung mechanics. That study had been based on the results of 27 experiments on anæsthetized sheep. Lung compliance and resistance to air flow were measured during spontaneous respiration from the simultaneous recording of air-flow rate, tidal volume and intrapleural pressure. The changes in resistance to air flow were insignificant, except when a large amount of fluid was given and simple mechanical blocking might be expected. In all cases water produced a gross fall in lung compliance. Five minutes after the introduction of 1 ml. per kilogram of sea water or fresh water into the trachea, the lung compliance had fallen to one-third of the control value. When prior vagotomy had been performed, compliance decreased 50%. Similar values had been found when the measurement was repeated half an hour after the introduction of the fluid; but after inflation of the lungs to 40 cm. of water pressure, there had been a marked increase in lung compliance only in those animals which had received fresh water. That difference had been related to the different absorption rates for sea and fresh water. The results obtained, together with those from other experiments, indicated that the compliance fall depended on the wide distribution, rather than on the volume of fluid administered, and that normal compliance could not be restored whilst even a small amount of water remained in the lung. The high surface tension of a water film probably produced closure of terminal airways, the fall in compliance being the result of the reduced amount of lung participating in expansion. Hypoxia following fluid was related to the fall in lung compliance, and was due to the passage of venous blood through non-ventilated parts of the lung. Vagotomy was found to have only a minor effect in protecting against the fluid-induced changes. A method of resuscitation had been based on the altered lung mechanics revealed in the experiments. It consisted essentially of intermittent inflation of the lungs to a maximum pressure of 30 mm. of mercury with 100% oxygen. When tested on sheep, that method allowed normal arterial oxygen saturation to be achieved, even when the hypoxia resulting from fluid had caused respiratory arrest. In conclusion, Dr. Colebatch said that the findings appeared to be applicable not only to drowning, but also to humans who had aspirated fluids. Because a similar alteration in lung mechanics had been described in pulmonary œdema, a similar approach to its control might be of value.

Alan Wynn, in a paper entitled "Emotional Stress in Relation to Heart Disease", said that the mortality from coronary disease was much higher amongst general practitioners than among consultants in the 25% of the English medical profession who were members of the Medical Sickness Annuity and Life Assurance Society. That difference had been attributed to the general practitioner's strenuous life. It could be explained, at least in part, by a subnormal rate of coronary disease in the consultants owing to a relative immunity of their cardio-vascular systems to degenerative processes; that they might inherit, together with the higher intellectual faculties which made them consultants. To test the theory, the age of death of the Fellows of the Royal Society obtained from the Biographical Memoirs for the years 1949–1956 were studied. Election to the fellowship depended almost entirely on intellectual eminence in science. The average age of death of the 148 Fellows was 76·0 years, and the average age of election 49·9 years. The average age of death of the male population of the United Kingdom, excluding those who died under the age of 50 years, was approximately 72 years in that period, so that the Fellows survived four years longer. They thus demonstrated a greater resistance to atherosclerosis, which was the cause of most of the deaths in that period. However, that a strenuous life might be a factor in cardio-vascular mortality from atherosclerosis was shown by an increased incidence of death from that cause in 1000 consecutive prominent white American men obtained from the obituary column of *Time Magazine* for the years 1949–1958. Men whose fame seemed due to social position or an isolated exploit were excluded; the remainder were thought to have been submitted to a greater intensity of nervous stimulation, irrespective of variation in quality of pattern, than the normal population. Their average age of death from cardio-vascular disease was 65 years—four to six years less than the white male population of the United States of America after the mortality under the arbitrary age level of 35 years had been excluded. The average age of death from non-cardio-vascular disease was reduced by a similar amount. That suggested that the study group was not typical of famous men, but included some men who had achieved additional fame by premature death, and excluded some whose fame had been diminished by longevity. It was considered that that source of error did not explain the whole difference, and that some of it might be due to the damaging effect of prolonged emotional tension upon the cardio-vascular and other systems of the body.

CLINICAL MEETINGS

A clinical meeting was held at the Royal Melbourne Hospital on Thursday, May 26. The following contributions were given: "Pyrexia of Unknown Origin", by C. H. Fitts; "Pulmonary Arterio-Venous Shunts Occurring in a Patient with Cirrhosis of the Liver: A Physiological and Pathological Study", by Professor C. R. B. Blackburn; "Cushing's Syndrome", the clinical features being presented by P. J. Parsons and laboratory aspects by Bryan Hudson; and "Essential Hyperlipæmia and Hypercholesterolæmia", by P. J. Nestel.

A second clinical meeting was held on Saturday, May 28, at St. Vincent's Hospital. The following contributions were given: "Polycythæmia: Hypercholesterolæmia and the Nephrotic Syndrome", by J. F. Niall; "Polycythæmia Associated with Chronic Fibrotic Tuberculosis", by H. I. Jones and I. S. Epstein, and presented by I. S. Epstein; "Clinical Observations on Neurological Manifestations in Acute Leukæmia", by J. H. Colebatch; and "Hypoparathyroidism and Hypothyroidism", by W. Hamilton Smith.

OFFICE BEARERS

The following is the constitution of Council for the period 1960–1961 :

President : T. M. Greenaway.

Vice-Presidents : F. Ray Hone, H. Maynard Rennie and E. H. Roche (New Zealand).

Censor-in-Chief : K. B. Noad.

Honorary Secretary : R. L. Harris.

Honorary Treasurer : Bruce Hall.

Past President : Professor J. G. Hayden.

Councillors : Fellows : J. J. Billings, Professor C. R. B. Blackburn, M. E. Chinner, J. Eric Clarke, Professor Lorimer Dods, Clive Fitts, John Halliday, Bruce Hunt, C. G. McDonald, Sir William Morrow, O. Ellis Murphy, E. H. Roche, Morvyn Williams, and H. G. Wilson. Members : J. C. English and T. H. Hurley.

Executive Committee : T. M. Greenaway,[1] H. Maynard Rennie, K. B. Noad, R. L. Harris,[1] Bruce Hall,[1] Professor J. G. Hayden, Sir William Morrow, and O. Ellis Murphy.

Assistant to the Honorary Secretary : John R. Sands.

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Censor-in-Chief : K. B. Noad.

Australian Board : J. Mark Bonnin, Eric Clarke, J. L. Frew, W. E. King, Sir William Morrow, and F. Hales Wilson.

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[1] *Ex officio.*

COMMITTEES

Finance Advisory Committee

The present Finance Advisory Committee is constituted as follows : M. C. Alder, Esq., A.I.A., F.I.I.A. (Chairman), the President,[1] the Honorary Secretary,[1] the Honorary Treasurer,[1] Dr. C. G. McDonald, L. G. Oxby, Esq., F.I.A., A. H. Pollard, Esq., M.Sc., M.Sc. (Econ.), Ph.D., F.I.A.

Editorial Committee

The Editorial Committee of AUSTRALASIAN ANNALS OF MEDICINE for the period 1960–1962 is constituted as follows : C. G. McDonald (Chairman), R. R. Winton (Editor), Ralph Reader (Honorary Secretary), Professor C. R. B. Blackburn, Professor Sir Edward Ford, Bruce Hall,[1] R. L. Harris,[1] John Read, and H. M. Whyte.

The Editorial Representatives are as follows : M. K. Gray, J. O. Mercer, E. G. Sayers and Professor F. H. Smirk, of New Zealand ; Ian Mackerras and Professor John Tyrer, of Queensland ; Professor A. A. Abbie, Professor H. N. Robson and E. B. Sims, of South Australia ; J. L. Grove and Sir Ralph Whishaw, of Tasmania ; Cyril Fortune and Professor Eric Saint, of Western Australia ; Sir Macfarlane Burnet, Professor E. S. J. King, T. E. Lowe and A. J. M. Sinclair, of Victoria.

Library Committee

Dr. H. Maynard Rennie has been appointed Honorary Secretary of the Library Committee.

Dominion and State Committees

The Dominion and State Committees for the period 1960–1962 are as follows :

New Zealand : M. K. Gray (Chairman), Graham Riley (Honorary Secretary), R. H. Quentin Baxter, O. W. Chapman, J. A. K. Cuningham, C. Greeson, W. E. Henley, I. A. M. Prior, E. H. Roche,[1] J. M. Twhigg and Morvyn Williams.[1]

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Tasmania : J. L. Grove (Chairman:, L. W. Knight (Honorary Secretary), P. L. Dorney, T. S. Kirkland, K. S. Millinger and F. R. T. Stevens.

Victoria : J. Eric Clarke[1] (Chairman), Bryan Hudson (Honorary Secretary), I. S. Epstein (Assistant Honorary Secretary), D. A. Alexander, M. V. Clarke, J. H. Colebatch, D. G. Duffy, Clive Fitts,[1] J. M. Gardiner, H. W. Garlick, D. R. Gauld, K. J. Grice, G. C. de Gruchy, Professor J. G. Hayden,[1] T. H. Hurley,[1] A. V. Jackson, R. R. H. Lovell, A. C. Newell, M. J. Robinson and T. H. Steel.

Western Australia : Bruce Hunt (Chairman), Alex Cohen (Honorary Secretary), T. B. Cullity, Gordon Hislop, W. R. Pitney, Professor Eric Saint, Ian Wallman.

[1] *Ex officio.*

MEMBERSHIP

Admission of Fellows. The following Fellows were admitted on May 25, 1960, after election by the General Body of Fellows : under Article 44, F. W. A. Clements, of New South Wales ; Professor F. C. Courtice, of Canberra ; D. O. Shiels, of Victoria ; and Sir Geoffrey Todd, of Sussex, England ; under Article 42 : B. A. D. Curtin, Reay Eakin, E. J. Halliday, G. L. McDonald and John Sands, of New South Wales ; R. A. Burston, J. S. Coverton, A. Kerr Grant, Robert Hecker and John McPhie, of South Australia ; Alan V. Jackson, Herbert I. Jones, Luke Murphy, H. Pincus Taft and Howard E. Williams, of Victoria ; Paul Dorney and F. R. T. Stevens, of Tasmania ; G. F. Hall and W. J. Smith, of New Zealand.

Admission of Members. The following candidates were successful at examinations held in New Zealand on February 17, 1960: John N. Armour, Keitha Corlett, Peter N. Leslie, P. G. Lindsay, Josephine M. Lord, J. B. Lowe, Brian P. McLaurin, Desmond O. Oliver, W. M. Porteous, Arthur N. Turnbull, W. F. Watters and H. J. Weston. The following candidates were successful at an examination held in Melbourne on May 23, 1960: B. R. Beveridge and B. A. Kakulas, of Perth; K. R. Hayes and K. P. Kennedy, of Queensland; J. T. Andrews, G. T. Ey and A. J. Watson, of South Australia; H. C. Burger, Kevin Catt, B. S. Faragher, J. E. Fitzgerald, J. Hansky, A. K. Lethlean, I. G. Lyall, J. F. O'Callaghan, S. F. Phillips, J. G. Sloman and R. R. W. Townley, of Victoria; O. Appenzeller, A. G. G. Bennett, L. Brenner, D. S. Child, John M. Duggan, N. D. Gallagher, B. Haneman, A. M. Lloyd, M. S. Owen, B. J. Pascoe, R. A. Royle, J. D. Wingfield and G. M. Watson, of New South Wales; K. F. Anderson, of South Australia; and Saul Wiener, of Victoria, were admitted to Membership under the provisions of Article 37.

Honour. The honour of Commander of the Most Excellent Order of the British Empire has been bestowed by Her Majesty the Queen upon Dr. H. McLorinan.

Obituary. The Council records with regret the deaths of Dr. J. R. Boyd, of Eastborne, New Zealand; Professor W. Keith Inglis and Dr. C. H. Shearman, of Sydney, who were Foundation Fellows of the College; Dr. R. C. E. Brodie, of Melbourne, and Dr. M. W. Fletcher, of Launceston, who were Fellows of the College; Dr. C. B. Berryman, of Melbourne, Dr. M. D. H. Harpur, of Sydney, and Dr. J. T. Paton, of Orange, New South Wales, who were Members of the College.

Membership. The College now has a roll of 11 honorary Fellows, 364 Fellows and 623 Members.

GENERAL

The Collier Trust Fund. A donation of £500 from the Collier Trust was received with appreciation. This makes a total of £1000 available from this trust for use by the College, under the terms of the gift, for medical education in Australia. It is proposed to award grants-in-aid to enable members of the College requiring such assistance to visit other institutes in the Commonwealth.

Rowden White Medical Education Fund. The terms of the benefaction of £1000 made by Dr. A. E. Rowden White, C.M.G., M.D., F.R.A.C.P., in 1944, have recently been altered in accordance with his wishes, so that when the Fund accumulates to the sum of £5000 the income will be used to provide for higher medical education for younger members of the College.

Examination for Membership. The Royal College of Physicians of London has decided that members of The Royal Australasian College of Physicians may, if they apply to the London College, be granted partial exemption from examination for Membership of that College. The Council of this College has agreed to a similar concession being made to members of the London College, and it is expected that the reciprocal arrangement will be introduced in 1961.

Jubilee Fund. The present total of promised donations to the Jubilee Fund now amounts to the sum of £65,000. The outstanding success of this Fund will prove of great value to the College.

Future Meetings of the College. The venue of future meetings of the College is as follows: 1960, ordinary meeting, Sydney; 1961, annual meeting, Perth, and ordinary meeting, Melbourne; 1962, ordinary meeting, Canberra; 1963, annual meeting, Sydney, and ordinary meeting, Brisbane; 1964, annual meeting, New Zealand.

Pfizer Travelling Fellow. The Pfizer Travelling Fellowship for 1961 has been awarded to Dr. E. J. Halliday, of Sydney.

Sims Travelling Professor. Professor John McMichael, M.D., F.R.C.P., F.R.S., Professor of Medicine at the Postgraduate Medical School of London, visited Australia and New Zealand as Sir Arthur Sims Commonwealth Travelling Professor from April to June, 1960. The appointments of Sir Arthur Sims Travelling Professors for 1961 have been announced as follows: H. J. B. Atkins, Esq., M.Ch. (Oxon), D.M., F.R.C.S., and Professor T. C. Gray, M.D., F.F.A.R.C.S., D.A., of England, to visit Australia and New Zealand, and Dr. P. H. Wood, M.D., F.R.C.P., of England, to visit Canada.

Representatives of the College. The following have been appointed to represent the College: the President, Dr. T. M. Greenaway, the Honorary Secretary, Dr. R. L. Harris, of Sydney, Professor J. G. Hayden, of Melbourne, and Dr. K. B. Noad, of Sydney, on the Joint Advisory Committee with the Royal Australasian College of Surgeons; Sir William Morrow, of Sydney, at the Conference on Post-Graduate Medical Education, to be held in Sydney in August, 1960; Sir Charles Burns, of Wellington, on the Committee of Inquiry on the Availability and Distribution of Medical Practitioners in New Zealand, and also on the Wellington Hospital Medical Appointments Advisory Committee, and Dr. Wilton Henley, of Auckland, on the Auckland Hospital Medical Library Committee.

Gifts to Library. Books for the library have been received with appreciation from the following: the Victorian Branch Library of the British Medical Association and the Medical History Museum of the Victorian Branch of the British Medical Association; Professor J. B. Cleland; Miss Evans; Dr. W. E. L. H. Crowther; the Institute of Medical Research, The Royal North Shore Hospital of Sydney; Dr. R. Jeremy; Mrs. F. H. Lack; Mrs. Arthur Moseley; Sir John Parkinson; Dr. H. Maynard Rennie; Mrs. Ruby Rich Schalit.

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